REMARKS

Claims 1, 2, 4-15, 17-19, 21, 30, 31 and 33 remain pending in the application. Claim 34 has been cancelled herein. No other amendments have been made.

Method claims 18, 21, 30 and 33 have been rejected as indefinite for, inter alia, being "of indeterminate scope." The Examiner further asserts that "the claim language may read on disorders not yet known to be associated with serotonin 5 HT receptors…." Applicants do not understand how, particularly given the prosecution history of this application family, these can be considered bases for rejection of the present claims. It is also noted that method claim 19, reciting a substantial list of specific disorders that can be considered to fall under the umbrella of "5-HT-mediated," has not been made a part of this rejection.

As is clear from the instant disclosure, the basis for the claimed invention is that the inventive compounds have selective affinity for 5-HT_{1B} receptors and thus would have a significant effect on 5-HT-mediated neurotransmission. The current knowledge in the field provides high expectation of the treatability of the recited diseases by the instantly claimed compounds. From this knowledge and the instant disclosure, the expectation would be that all of the disorders recited are, at least in some instances, mediated by 5-hydroxytryptamine. Thus,

the instant compounds would be expected to be effective in the treatment of any disorders affected by 5-HT_{1B} receptor antagonists. It is neither necessary nor appropriate that a particular disorder be recited, nor is it of any relevance to patentability whether the entire list of disorders that fall into this category is presently known.

One of the reasons that the Examiner has alleged that these claims are indefinite is that they contain the language "in need of such treatment." Applicants wish to point out that the language in question is a convention, the standard claim language used in method-of-treatment claims. It is not by any means required, and, in fact, is actually redundant in a sense; it should be clear to anyone reading such claims that the inventors were not contemplating treating patients that had no need of the treatment in the first place. And, contrary to the Examiner's implication, candidates who might benefit from treatment would be readily identifiable. In any event, the question of determining who needs treatment and who does not should not be of relevance to the determination of patentability in this context. Is the Examiner suggesting that deletion of the language would be remedial?

In trying to justify this "in need of" prong of the rejection, the Examiner asserts that "5-HT receptors may be involved in all diseases…." Clearly, this cannot be the case,

and it appears that the Examiner is trying to play "devil's advocate." This is not a proper basis either for leveling a rejection.

The Examiner further asserts that "determining whether a given disease responds or not to such a mode of action involves much experimentation..." and questions what success rate determines if a particular drug is effective. These are inappropriate considerations to invoke in the consideration of patentability. Demonstration of patentability does not require demonstrating the curing of a disease in a patient or a "threshold" number of patients; it merely requires providing some support, that one of skill in the art would find credible, for the assertion that the compounds in question could be effective in the treatment of the recited diseases. In grandparent application Serial No. 09/171,575, now U.S. Patent No. 6,548,498, Applicants provided just such support. This support is also presented again later in this communication.

Applicants note further that claims of the identical form of instant claims 18, 21, 30 and 33 can be found in the above-mentioned '498 patent. There is nothing above the particular compounds recited in the instant method claims that renders these claims any more liable to an indefiniteness rejection than were the method claims of identical form reciting the compounds found in the '498 patent. In fact, all variables in formula I

of instant claim 1 are, except for variable X, defined identically to their counterparts in formula I of claim 1 of the '498 patent. This rejection is arbitrary and should be withdrawn.

The Examiner asserts that claims 18 and 21 are substantially duplicates. This assertion is, among other things, inconsistent with the Examiner's own [incredible] assertion, cited above, that "5-HT receptors may be involved in all diseases." In any case, while this statement is certainly not true, it is certainly reasonable to suppose that not all 5-HT-mediated disorders occur solely in the central nervous system (note, for example, the application of the instant compounds to the inhibition of tumor growth). Again, Applicants note that claims of identical scope to instant claims 18 and 21 with respect to the diseases to be treated can be found in the '498 patent.

Claim 34 has also been rejected as indefinite. The cancellation of this claim herein renders moot the rejection.

The claims have been rejected as not being enabled.

In the first place, the Examiner asserts that the "[S]cope of 'solvates' which is embraced by all the claims is not remotely enabled...." The Examiner refers to "the teachings of the specification which particularly describe only hydrates." It should be pointed out first that hydrates are disclosed on

page 17 of the specification as being the "preferred solvates"; Applicants should not be penalized for disclosing a preferred embodiment by being required to limit their claims to that embodiment. Secondly, one of skill in the art would clearly understand that not all solvents can form solvates with all compounds. The language of the compounds, which is standard, would clearly be understood by anyone of skill in the art to encompass those compounds for which solvation is possible.

The Examiner goes on to assert that the specification disclosure of an assay for screening compounds with the desired properties does not enable the various uses recited in the method claims. In the first place, Applicants provide below data determined by the disclosed assay which demonstrates the efficacy of the compounds of the present invention. Again, these data were originally presented during prosecution of application Serial No. 09/171,575 (the grandparent of the present application), now U.S. Patent No. 6,548,498.

The compound of Example 4 of the instant specification was tested for its affinities for the 5-HT_{1A} and gp5-HT_{1B} (previously known as 5-$HT_{1D\beta}$) receptors. The method for determining 5-HT_{1A} receptor binding affinity was that of Jackson, et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 351: 146-155 (1995).

Binding affinity for the 5-HT_{1B} receptor was determined by measuring [^{3}H]GR125743 binding to guinea pig cortical 5-$HT_{1B/D}$ receptors as follows:

Preparation of membranes:

Duncan-Hartley guinea pigs weighing 300-400 g (obtained from Charles River, Uppsala, Sweden) were housed under standard laboratory conditions (18-22^{0}C, 40-80% humidity, 15-18 air changes/h, artificial 12/12 dark/light cycle, lights on at 6:00) with free access to food (K1 from Lactamin; Stockholm, Sweden) and tap water. The guinea pigs were decapitated and the cortices dissected out, weighed and homogenized in 50 mM Tris-HCl, pH 7.7 with an Ultra-Turrax followed by centrifugation for 10 min at 48,000 x g and 5^{0}C. The pellet was resuspended and recentrifuged. The final pellet was suspended in 0.32 M sucrose buffer to a concentration of 0.5 g original wet weight per mL, and stored frozen at -70^{0}C.

Radioligand binding assays:

[^{3}H] GR125743 saturation studies were carried out in duplicate with 3-4 mg w.w. per tube in 5 ml buffer (50 mM Tris, 4 mM $CaCl_2$, 4 mM $MgCl_2$ and 1 mM EDTA at pH 7.7), and a concentration range of 0.012-2 nM (10-12 conc.) for the radioligand. Nonspecific binding was determined in the presence of 10μM methiothepin.

In the competition experiments, 4-8 mg w.w. per tube and a radioligand concentration of 0.2 nM were used with 10-12 concentrations of the competing drug. The assays were run for 2-4 hours at 30°C, and terminated by rapid filtration through Whatman GF/B filters (pretreated with 0.1% polyethyleneimine) using a Brandel cell harvester. Bovine serum albumin (0.1%) was added to the washing buffer to reduce nonspecific binding.

Data from the experiments were analyzed using the iterative nonlinear curve-fitting program LIGAND (Munson, et al., Anal. Biochem. 107: 220-239 (1980)). The K_d value obtained from the saturation studies was used in the calculation of the K_i values by the LIGAND program. The K_d value of [^{3}H] GR125743 was determined to be 46 ± 4 pM and the B_{max} value to be 4.9 ± 0.2 pmol/g w.w.

The results of testing of the compound according to the invention are shown in the following table:

	K_i, 5-HT_{1A} (nM)	K_i, $_{gp}$5-HT_{1B} (nM)
Example 4	1296	1.0

As can be seen from the above data, the test compound exhibited significant selectivity for the 5-HT_{1B} receptor. The compound of

instant Example 4 showed a 1296-fold affinity for the 5-HT_{1B} receptor over the 5-HT_{1A} receptor.

The Examiner cites various criteria for enablement as set out in *In re* Wands and asserts that the rejected claims do not meet these criteria.

The data originally provided in the grandparent application, and again immediately above, address the Examiner's expressed concern that the "amount of guidance presently in the specification as to which compounds are sufficiently active... is nonexistent." The data also render irrelevant The Examiner's comment regarding the dosage range information provided in the specification. These test data also address the Examiner's expressed concern regarding the working examples and lack of test data.

With respect to the issue of level of skill in the art, Applicants wish to point out that, contrary to the Examiner's assertion, there **is** "evidence that the current state of the art is one in which 5-HT1B agonists/antagonist have such a range of uses." The Examiner refers to, for example, the review article of Halazy, et al., previously cited by Applicants, as being among those not providing the required evidence. However, the Examiner is mistaken in this assessment. Throughout both this publication and that of Göthert, et al. also cited by Applicants

in the previously submitted Information Disclosure Statement, reasons can be found, based on the experimental work performed thus far, for high expectation of the wide efficacy of compounds such as those of the instant invention. As reported in these reviews, there is an intimate correlation between the h5-HT_{1B} receptor and the indications recited in the instant claims.

The Examiner's attention is particularly directed to Halazy, page 349, second column, last two paragraphs and Göthert, from the last 16 lines of page 337 to the middle of page 339. Both passages particularly point out the probability of the involvement of 5-HT and 5-HT autoreceptors in disorders such as those recited in the instant claims, as well as the probability of effectiveness of 5-HT_{1B} receptor antagonists in the treatment of such disorders. Thus, one of skill in the art would have reasonable expectation that the compounds of the instant invention, with their affinity for 5-HT_{1B} receptors, would be effective in the treatment of the disorders recited in the instant method claims.

Further with respect to the Göthert reference, the Examiner indicated in the present Office Action that this reference was not considered because the Examiner was unable to obtain the parent files containing the reference and the IDS filed in the instant application did not provide sufficient citation information. Accordingly, Applicants have provided herewith for

the Examiner's convenience another copy of the Göthert reference and ask that it be given full consideration as support for the idea that the scope of recited diseases is enabled. It appears from her invocation of the Halazy reference in the Office Action that the Examiner may already have access to this reference. Nonetheless, to be sure, Applicants also provide herewith for the Examiner's convenience a copy of Halazy.

Further with respect to the data provided herein, Applicants anticipate that the Examiner may note that the tested compound is not actually a member of the group of compounds being claimed in the present application, which group is a subgenus of the originally claimed genus of compounds. It must be emphasized that the segregation of the instantly claimed compounds from those allowed in the '498 patent is not by any means a proof or even an indication that the respective groups of compounds would be expected to have different properties. The reason for the segregation of compounds was the imposition of a restriction requirement in the grandparent application. As argued forcefully but to no avail by Applicants in the grandparent application, the restriction of compounds was an arbitrary act and created on artificial division that was neither based on proper application of PCT Unity of Invention criteria, nor based on any sound scientific principles or information. Again, all variables in formula I of instant claim

1 are, except for variable X, defined identically to their counterparts in formula I of claim 1 of the '498 patent.

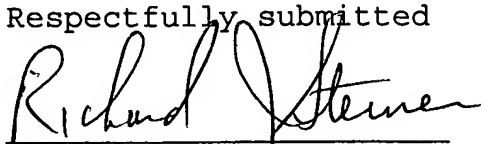
There is no valid reason to suppose that the compounds presently being claimed, compounds which are closely related to the test compound, would not have the same receptor specificity as the test compound. Applicants have provided compelling data showing the high selectivity of a representative compound from the genus of compounds originally claimed and should not be penalized in this instance by the previous arbitrary assessment that the instant compounds are patentably distinct from the compounds allowed in the grandparent application and hence, by implication, might be expected to have different properties from said allowed compounds.

The arguments and test data set forth above fully address the Examiner's expressed concerns regarding the definiteness and enablement of the instant claims. Reconsideration and allowance of the application with pending claims 1, 2, 4-15, 17-19, 21, 30, 31 and 33 are respectfully requested. Should any other matters require attention prior to allowance, it is requested that the Examiner contact the undersigned.

The Commissioner is hereby authorized to charge any other fee which may be due for any reason in connection with this communication to Deposit Account No. 23-1703.

Dated: July 14, 2005

Respectfully submitted



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Enclosures

Review

Central & Peripheral Nervous Systems

5-HT1B/1D antagonists and depression

Serge Halazy, Marie Lamothe & Catherine Jorand-Lebrun

The molecular pharmacology, functional role and behavioural implications of central 5-HT1B/1D receptors suggest that these particular receptor subtypes probably play an important role in brain serotonin neurotransmission. This article summarises the rationale for considering 5-HT1B/1D antagonists as potential new, fast-acting antidepressants and describes the development of recent selective potent 5-HT1B/1D antagonists, reviewing both the primary and patent literature.

Exp. Opin. Ther. Patents (1997) 7(4):339–352

1. Introduction

Serotonergic disturbance in depressive illness is now well established [1] and several lines of preclinical and clinical evidence indicate that an enhancement of 5-HT mediated neurotransmission might underlie the therapeutic effect of most antidepressant drugs [2]. Among them, selective serotonin re-uptake inhibitors (SSRIs), for example fluoxetine, fluvoxamine, sertraline, citalopram and paroxetine, have been shown to be clearly effective in the treatment of major depression. Nevertheless, the earliest signs of therapeutic improvement in depressive symptoms only appear after about two weeks and the clinical efficacy of SSRIs does not exceed a maximum effect of 60 - 75% [3,4].

It appears paradoxical that SSRIs inhibit the 5-HT re-uptake site within minutes of administration, whereas their full antidepressant effect (improvement in mood) requires several weeks to appear. As reported on many occasions, this observation clearly indicates that re-uptake inhibition *per se* is not responsible for the antidepressant responses, but rather that adaptive changes underlie the therapeutic effects of SSRIs [2,5]. In other words, the antidepressant effect of SSRIs involves subsequent adaptations of other mechanisms that regulate serotoninergic neurotransmission.

Among the different hypotheses (for example, down-regulation of somatodendritic 5-HT1A or post-synaptic 5-HT2 receptors) proposed to explain the delayed onset of the therapeutic action of SSRIs, the down-regulation of terminal autoreceptors (5-HT1B/1D receptor subtypes) appears to be particularly attractive since several lines of evidence connect 5-HT1B/1D receptor function with the pathophysiology of depression. Firstly, 5-HT1B/1D autoreceptors in serotonin terminals suppress the synthesis [6] and release [7] of serotonin in rat brain (thus decreasing serotonin neurotransmission). Moreover, both auto- and hetero-5-HT1B presynaptic receptors have been shown to be desensitised after the long-term treatment with antidepressants [5,8,9] and it has been reported that 5-HT1B receptor stimulation blocks the antidepressant-like effects of SSRIs in animal models of depression [10,11]. Very recent studies [12] have shown that chronic fluoxetine reduces the level of serotonin transporter mRNA and 5-HT1B mRNA in a sequential manner in the rat dorsal raphe nucleus, suggesting that chronic fluoxetine may increase serotonin release from axonal terminals mainly by down-regulating the expression of the messenger RNA coding for presynaptic 5-HT1B autoreceptors.

Thus, the delayed onset of therapeutic action of SSRIs is believed to be due to the time required for desensitisation of (5-HT1B/1D-type) autoreceptors. As a consequence, the blockade of terminal 5-HT1B/1D receptors by selective antagonists has been proposed [5,13] as a new approach toward the design of potentially more efficient and/or fast-acting antidepressant drugs since acute 5-HT1B/1D receptor blockade would in theory immediately elevate terminal 5-HT release. For the same reasons, it is anticipated that the combination of

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an SSRI with a 5-HT1B/1D antagonist will shorten the onset of the antidepressant action of an SSRI. Finally, recent studies suggest that supersensitive 5-HT1B/1D receptors may be involved in the pathophysiology of obsessive compulsive disorders (OCD) supporting the hypothesis that selective 5-HT1B/1D antagonists may also represent potential drugs for its treatment [14].

2. 5-HT1B/1D receptors

The 5-HT1B binding site was first characterised in rat brain membranes and the 5-HT1D binding site in bovine caudate nucleus [15]. Molecular biological studies later demonstrated that the human 5-HT1D receptor site is encoded by a family of two distinct genes, termed initially 5-HT1Dα and 5-HT1Dβ [16]. Binding studies have previously shown that the human and rat receptors were 'pharmacologically different', as demonstrated, for example, by studies with propanolol which binds to the rodent 5-HT1B receptor with nanomolar affinities but recognises 5-HT1Dβ binding sites only very poorly (IC_{50} > 1000 nM) [17].

It has now been demonstrated that this difference in pharmacological profile is the result of a single amino acid modification [18,19]. Since the amino-acid sequence, the function and the regional distribution of the rodent 5-HT1D receptor and of the human 5-HT1Dβ receptor are nearly identical, these two receptors are considered as species homologues. Thus, the Serotonin Club Nomenclature Committee has recently proposed a revised nomenclature for 5-HT1B, 5-HT1Dα and 5-HT1Dβ receptor subtypes [20]. 5-HT1Dα receptors are now termed 5-HT1D and 5-HT1Dβ receptors are now termed 5-HT1B with distinction between human (h5-HT1B) and rat (r5-HT1B) receptors, to account for their pharmacological differences.

5-HT1B/1D receptors belong to the family of seven transmembrane G protein-coupled receptors (both coupled to adenylate cyclase) and are implicated in important functional activities [21]. For example, presynaptic 5-HT1B/1D receptors on serotonergic axon terminals control the release of 5-HT while 5-HT1B/1D heteroceptors control the release of other neurotransmitters (GABA, glutamate, acetylcholine, dopamine, noradrenaline) from non-serotonergic terminals, while yet others are situated postsynaptically on the target neurones of 5-HT projections. In the periphery, activation of 5-HT1B/1D receptors on blood vessels leads to vasoconstriction [22] and inhibition of protein extravasation [23], two mechanisms relevant to the antimigraine activity of 5-HT1B/1D agonists such as sumatriptan, naratriptan, zolmitriptan or rizatriptan.

3. Pharmacological evaluation of 5-HT1B/1D antagonists

Cloning and expression of human 5-HT1B and 5-HT1D receptor subtypes in different cell lines offers powerful tools to identify and characterise human 5-HT1B/1D antagonists, not only because of the importance of working with human receptors, but also because these models rapidly give access to relevant properties as binding affinity and more importantly, intrinsic activity. Thus, at the cellular level, 5-HT1B/1D antagonists can be characterised as molecules having no intrinsic activity and the ability to antagonise an agonist-induced response for the chosen receptor subtype. Such data can be obtained by measuring the potency of an antagonist either to:

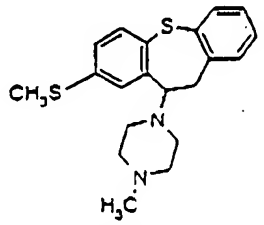
- block the inhibition of forskolin-mediated adenylate cyclase induced by an agonist [24]
- antagonise stimulated [^{35}S]-GTPγS binding [25]
- inhibit cell growth promoted by receptor stimulation with an agonist [26]

These different models are thus particularly useful to identify potent silent and selective human 5-HT1B/1D antagonists. Further *in vitro* pharmacological models currently used to characterise 5HT1B/1D antagonists include inhibition of agonist-induced contractions of saphenous vein in dogs [27] and rabbits [28] and 5-HT release experiments with guinea-pig cortex or hypothalamus slices in which blockade of 5-HT release induced by a 5-HT1B/1D agonist can be prevented by an antagonist [29].

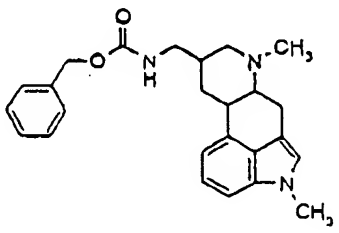
In vivo models relevant to the characterisation of 5-HT1B/1D antagonists and functional neuropharmacology of compounds acting at 5-HT1B/1D receptors have been recently reviewed [30,31]. It is worth mentioning here that blockade of hypothermia in the guinea-pig induced by stimulation of 5-HT1B/1D receptors with an agonist (for example GR 46611 [32] or SKF 99101H [33]) is also widely studied to characterise 5-HT1B/1D antagonists *in vivo*. However, although this particular model may find utility to assess brain penetration or bioavailability of potential antagonists, the data have to be exploited carefully since recent studies [33] suggest that hypothermia is likely to be mediated by postsynaptic receptors rather than by presynaptic 5-HT1B/1D autoreceptors located on serotonergic neurones.

Interestingly, the enhancement of 5-HT neurotransmission, (which is the rationale for the potential use of 5-HT1B/1D antagonists as antidepressants) can be monitored in freely-moving guinea-pigs by measuring the extracellular levels of 5-HT in brain terminal areas by microdialysis [5]. This technique allows the meas-

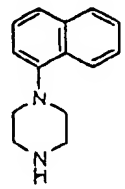
Figure 1: Non-selective antagonists of the 5-$HT_{1B/1D}$ receptor.



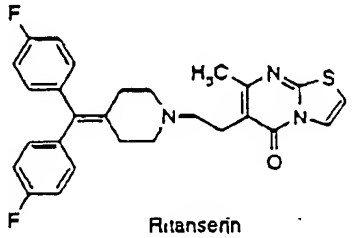
Methiotepin



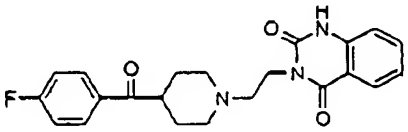
Metergoline



1-Naphthylpiperazine



Ritanserin



Ketanserin

Table 1: Binding affinities (pK_i) at human cloned receptor.

	5-HT_{1A}[§]	5-HT_{1B}[§]	5-HT_{1D}[§]	5-HT_2[§§]
Methiotepin	7.7	8.9	9.2	8.8
Metergoline	8.5	8.3	8.8	9
1-Naphylpiperazine	8.0	8.0	8.2	-
Ritanserin	6.1	7.2	8.4	8.8
Ketanserin	5.5	5.8	7.9	8.7

[§]Values determined at human cloned receptors.
[§§]Values for binding at 5-HT_2 recognition sites in mammalian brain.

urement of the effect of a 5-$HT_{1B/1D}$ antagonist alone on brain 5-HT levels or the effect of an antagonist on agonist-induced inhibition of 5-HT release.

4. 5-$HT_{1B/1D}$ antagonists

4.1 Non-selective pharmacological tools

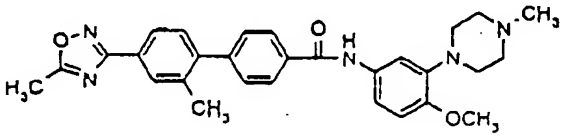
Until recently, pharmacological studies of 5-$HT_{1B/1D}$ receptors have mainly been performed with non-selective antagonists e.g., methiothepin, metergoline or 1-naphtylpiperazine (1-NP) [34] which block most 5-HT_1 and 5-HT_2 receptor subtypes (Figure 1)

Despite their lack of selectivity, such compounds have been particularly useful in assessing the role and importance of presynaptic 5-$HT_{1B/1D}$ receptors in the control of serotonin release. This is more particularly the case for methiothepin [35] which is a potent and silent antagonist at 5-$HT_{1B/1D}$ receptors subtypes (Table 1). More recent studies have shown that the well known 5 HT_2 receptor antagonists ketanserin and ritanserin are also potent and silent antagonists at human 5-HT_{1D} receptor subtypes (pK_B = 7.8 and 7, respectively) and among them, ketanserin appears as a useful pharmacological tool to distinguish between 5-HT_{1B} and 5-HT_{1D} receptor since this compound has almost no affinity (and no activity) at human 5-HT_{1B} receptors [36]. Thus, studies with ketanserin suggest that the serotonin-induced contraction of rabbit saphenous vein is mediated mostly by 5-HT_{1B} receptor subtypes [28], and, more importantly, that autoreceptors at axon terminals controlling 5-HT release are probably also 5-HT_{1B} receptors [37].

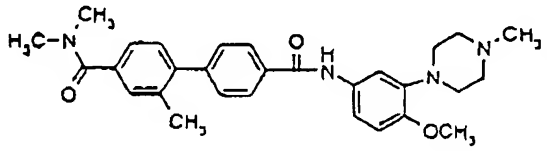
4.2 Selective 5-$HT_{1B/1D}$ antagonists

A major step in the process of functional characterisation of 5-$HT_{1B/1D}$ receptors has been the discovery of GR-127935 by researchers at Glaxo [101]. This new benzanilide derivative was reported in 1993 as the first example of a selective 5-$HT_{1B/1D}$ antagonist. The

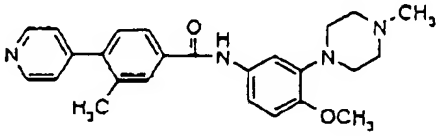
Figure 2: Selective antagonists of 5-HT$_{1B/1D}$ receptors.



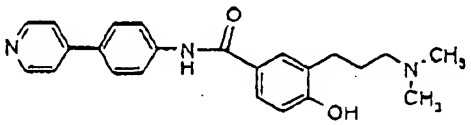
GR-127935



GR-133867



GR-125743



GR-55562

pharmacological properties of that particular compound have been extensively studied and summarised in a recent review [38] (Figure 2).

Controversial data have been reported concerning the ability of GR-127935 to increase synaptic 5-HT levels [38]. In the majority of the cases, it appears clear that this particular compound does not significantly enhance the synaptic 5-HT concentration *in vivo* upon systemic administration. Interestingly enough, more recent investigations, especially at the level of human cloned 5-HT$_{1B/1D}$ receptors, have shown that GR-127935 acts as a weak but full agonist at 5-HT$_{1D}$ sites [39] and thus can be classified as a 'non-silent' antagonist (partial agonist) at 5-HT$_{1B}$ sites [38,40]. It has been postulated that the failure of GR-127935 to increase brain 5-HT release *in vivo* may be related to its intrinsic activity at 5-HT$_{1B/1D}$ receptor sites [21].

Several analogues of GR-127935 in which the 1-(5-amino-2-methoxyphenyl)-4-methylpiperazine pharmacophore has been kept intact have been prepared by Glaxo, for example GR-133867 and GR-125743, both of which bind to 5-HT$_{1B/1D}$ receptors with similar affinities to GR-127935 [102,103].

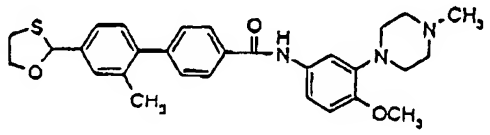
The hypothermia induced by SKF 99101 H in the guinea-pig can be blocked by GR-125743 to a similar extent as with GR-127935 [33]. Recent studies at the human cloned 5-HT$_{1B/1D}$ receptors show that both compounds have a very similar profile, showing non-negligible agonist activity at both receptor subtypes [41].

Further investigations from Glaxo reported in the patent literature concerning analogues of GR-127935 include replacement of the heterocyclic oxadiazole residue as illustrated by compound 1 (pK_i = 8.6 in the 5-HT$_{1D}$ guinea-pig striatum radioligand binding assay) [104], inversion of the amide bond (as in compound 2) [105], or replacement of the central amide bond by a methylene ketone linker, as exemplified by compound 3 [106]. The replacement of the piperazine moiety found in GR-127935 either by an amino alkyl side-chain or an alkyl substituted by a cyclic amine has been described in three specific patent applications [107-109] exemplified by compounds 4, 5 or 6. Further similar modifications of GR-127935 have also been claimed in two other patent applications [110,111].

It is noteworthy that, with the exception of compound 1, no precise biological data have been reported within these patent applications but, in all of them, compounds are claimed to inhibit 5-HT induced contractions of isolated dog saphenous vein and to antagonise the 5-HT induced inhibition of neurotransmission in central and peripheral neurones.

Fortunately, some interesting biological data concerning a few compounds which are part of the above-mentioned patent literature have been disclosed [42]. Reported data include affinity for guinea-pig 5-HT$_{1D}$ sites, antagonism of 5-HT-induced contraction of the dog saphenous vein *in vitro*, and *in vivo* antagonism of guinea-pig hypothermia induced by GR-46611 as illustrated with a few examples in Table 2. These results clearly show the advantage of replacing the 4-pyridinylphenyl system by a 4-substituted diphenyl moiety. On the basis of antagonism potency in the dog saphenous vein model and in the *in vivo* model of hypothermia, GR-127935 and GR-133867 appear clearly as the most interesting compounds.

However, as mentioned above, it was later found that GR-127935 is not a silent antagonist at human 5-HT$_{1B/1D}$ sites. More recent investigations from Glaxo and others have shown that, unlike GR-127935, GR-55562 could be characterised as a silent, potent and selective h5-HT$_{1B}$ receptor antagonist [43]. However, so far no results have been disclosed concerning the ability of this particular compound to antagonise central presynaptic 5-HT$_{1B}$ receptors upon systemic administration.



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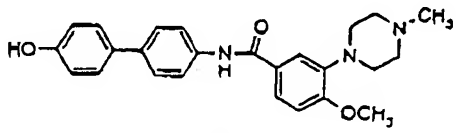

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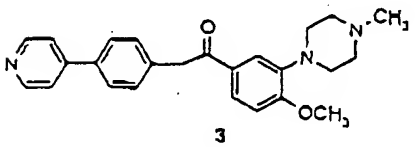

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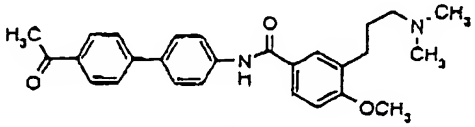

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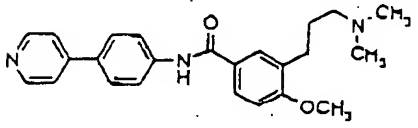

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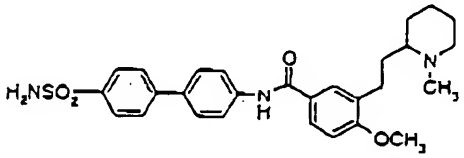

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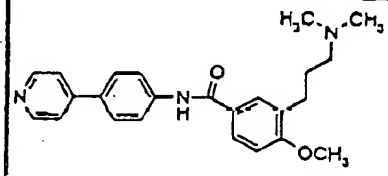
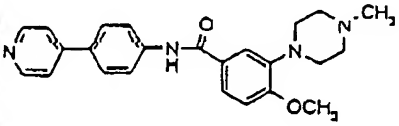
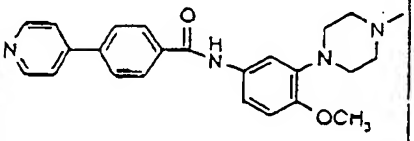
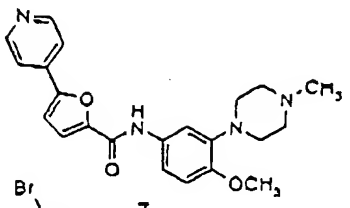
Table 2: Examples of data reported on 5-HT$_{1D}$ antagonists [42].

Compound	5-HT$_{1D}$ (pK$_i$)[a]	DSV (pK$_B$)[b]	Hypothermia ED$_{50}$ (mg/kg, p.o.)[c]
	8.0	7.8	> 30
	8.5	7.9	> 45
	8.3	8.0	5.0
GR-127935	8.5	-	0.3
GR-133867	8.3	9.2	0.67

[a]Affinity at guinea-pig striatum binding sites.
[b]Antagonism of 5-HT induced contraction of the DSV *in vitro*.
[c]Antagonism of hypothermia induced by GR-46611 in guinea-pig *in vivo*.
DSV: Dog saphenous vein.



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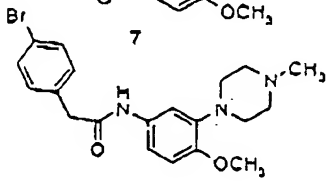

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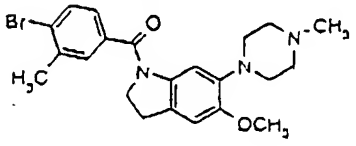

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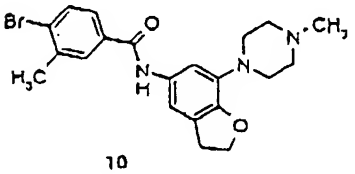

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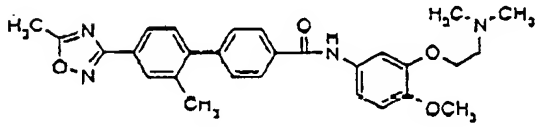

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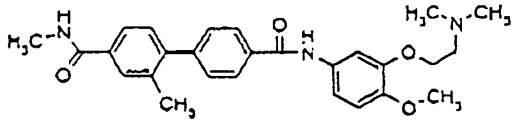

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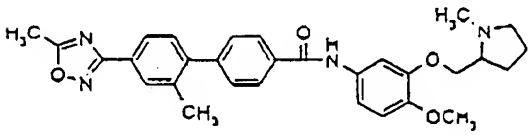

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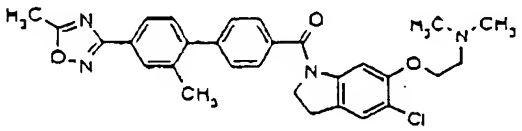

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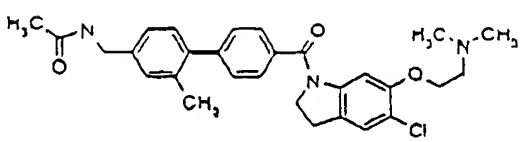

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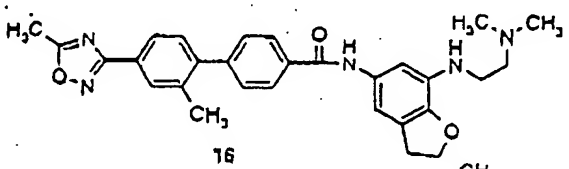
SmithKline Beecham has also disclosed a series of close analogues of GR-127935 which are derivatives of 1-(5- amino-2-methoxyphenyl)-4-methylpiperazine, for example compounds **7** and **8** [112,113]; clearly, these efforts have been mainly directed towards new analogues of GR-127935 in which the arylpiperazine moiety has been modified. The Company's first patents in the field claim fused bicyclic derivatives in which either the nitrogen atom of the amide bond or the oxygen atom of the methoxy group found in GR-127935 forms a fused cycle with the phenyl ring as exemplified by compounds **9** and **10**, respectively [114,115]. The piperazine ring of GR-127935 has also been replaced by an oxyethylamine residue as in compounds **11** [116] or **12** [117], or by an oxymethylene pyrrolidine as found in compound **13** [118]. Combination of the two structural modifications mentioned above in a single molecule resulted in patent applications in which compounds **14**, **15** and **16** are given as representative examples [119-121].

More recently, new analogues of GR-127935 in which the phenyl piperazine moiety has been modified into conformationally restricted fused or spiro ring systems have also been claimed as 5-HT1B/1D antagonists by SmithKline Beecham, as illustrated by compounds **17** [122], **18** [123] and **19** [124]. Finally, the piperidine derivative **20** which only differs from GR-127935 by the replacement of the piperazine with a piperidine ring has also been claimed by SmithKline Beecham [125] as one of many 5-HT1B/1D antagonists which are very close to compounds previously patented by Glaxo.

No biological information or pharmacological properties have been disclosed concerning compounds **7** to **20** in the above-cited patent applications. However, very recently, some interesting data concerning some of these compounds have been disclosed by SmithKline Beecham researchers at the occasion of a meeting on drug discovery [44]. The rational design of the SmithKline Beecham team was simultaneously based on the chemical structure of GR-127935, receptor molecular modelling studies on 3D putative structures of the 5-HT1B receptor and on structure-activity relationships built from data obtained with their own compounds tested at human cloned receptors (affinity and intrinsic activity).

Most of the SmithKline Beecham work has been focused on the modification of the *o*-methoxyphenyl piperazine substructure of GR-127935 with the aim to improve selectivity for 5-HT1B receptor subtypes (especially compared to 5-HT1D and 5-HT2 receptor sites) and, maybe more importantly, to gain access to 5-HT1B antagonists with no intrinsic activity. **Table 3** summarises a few selected examples illustrating how receptor



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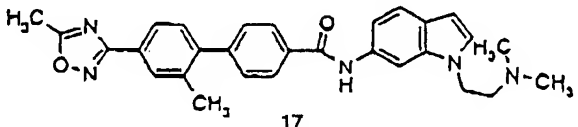

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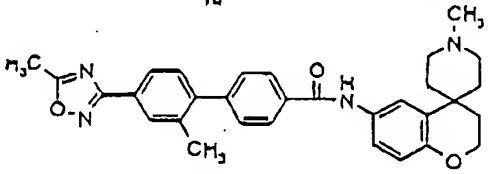

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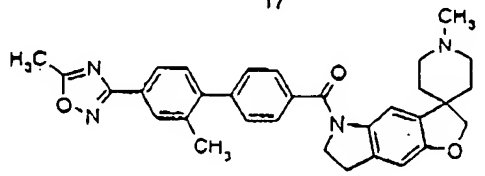

19 (SB-224289)

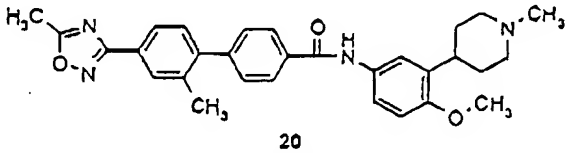

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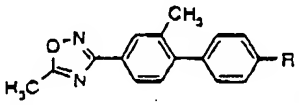
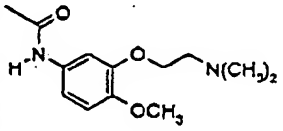
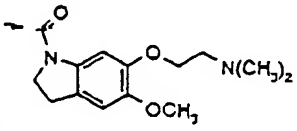
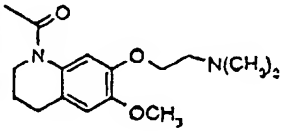
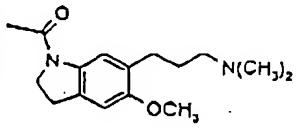
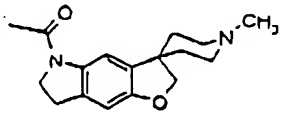
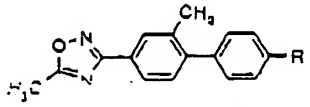
Table 3: Selectivity for 5-HT$_{1B}$ over 5-HT$_{1D}$ and 5-HT$_2$ receptors (pK$_i$) [44].

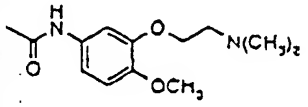
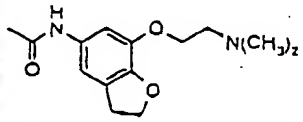
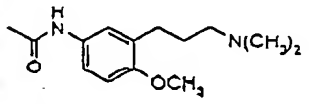
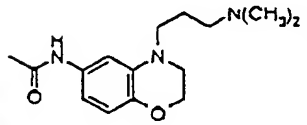


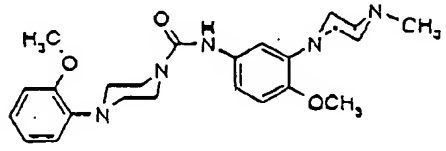
R	5-HT$_{1D}$	5-HT$_{1B}$	5-HT$_{2A}$	5-HT$_{2C}$
	NA	9.1	7.3	6.8
	NA	9.2	6.5	6.3
	NA	9.1	5.7	6.1
	7.0	8.5	NA	NA
	6.2	8.1	NA	NA

NA: Data not available.

Table 4: Agonist activity at 5-HT1B receptors [44].



R	pEC50	Emax (%)
	9.1	+ 38
	8.2	+ 70
	8.7	- 25
	7.0	0



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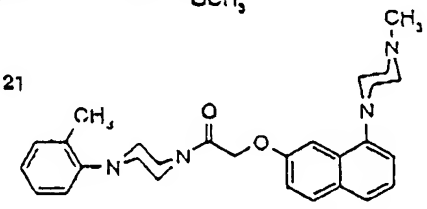

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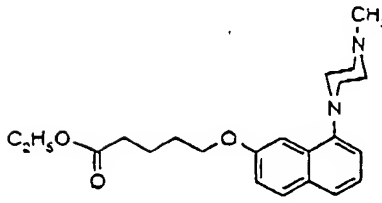

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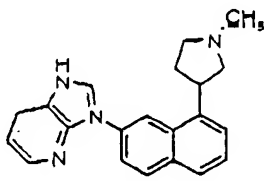

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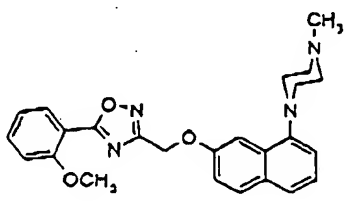
selectivity can be improved by conformational restriction around the amide bond or around the basic amino side-chain and **Table 4** illustrates the importance of the nature and the positioning of the basic amino side-chain on intrinsic (agonist) activity of these compounds since modifications at that level can discriminate between partial agonists, silent antagonists or even inverse agonists at 5-HT1B receptors. Altogether, it can be concluded that, starting from GR-127935, modifications of the piperazine ring can abort intrinsic activity and improve 1B *versus* 1D selectivity while conformational restriction of the amide bond can improve selectivity *versus* 5-HT2 receptors.

Combining all of these modifications in a single molecule resulted in the identification of SB-224289 which binds very selectively to 5-HT1B receptors (pK_i = 8.1) especially when compared to 5-HT1D (pK_i = 6.2), 5-HT1A (pK_i < 5.5), 5-HT1E (pK_i < 5), 5-HT1F (pK_i < 5) or 5-HT2 (pK_i < 6.2) sites.

SB-224289 appears to be an inverse agonist with negative intrinsic activity as demonstrated by using the GTPγS binding model in Chinese hamster ovary (CHO) cells expressing human cloned 5-HT1B receptors. In the same model, GR-127935 appears as a partial agonist. Interestingly, methiothepin was earlier suggested to be an inverse agonist on the basis of functional pharmacological data [35]. Antagonist properties of SB-224289 at 5-HT1B sites have also been evaluated in the *in vivo* model of 5-HT1B agonist-induced hypothermia (ED_{50} = 2.7 mg/kg) and, last, but not least, it has been disclosed that SB-224289, is able to antagonise the 5-HT1B agonist-induced inhibition of 5-HT release in the guinea-pig frontal cortex after oral administration (ED_{50} = 4 mg/kg). Thus, SB-224289 appears as the most interesting silent 5-HT1B antagonist (or inverse agonist) reported so far, and further pharmacological characterisation of that particular compound will be of primary importance to define the therapeutic potential of controlling 5-HT release by antagonising 5-HT1B/1D receptors in depression.

The replacement of the diphenyl moiety found in GR-127935 by an arylpiperazine residue has been claimed by Pierre Fabre Médicament to give selective, silent 5-HT1B antagonists, for example, compound 21, which binds to human 5-HT1B sites with an affinity of 2 nM while the EC_{50} is superior to 1000 nM (cAMP model in CHO-K1 cells transfected with human 5-HT1B receptors) [126]. Such compounds differ from GR-127935 by the absence of agonist intrinsic activity at 5-HT1B sites despite their high binding affinity and also by some binding selectivity between 5-HT1B and 5-HT1D receptor subtypes. These antagonists are also reported to inhibit the 5-HT-induced contraction of rabbit saphenous vein and to block the inhibition of



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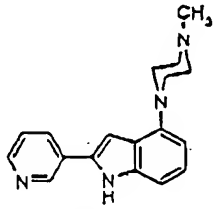

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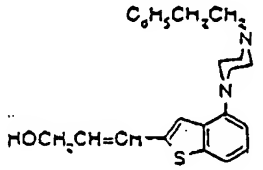

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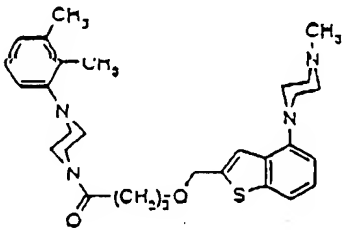

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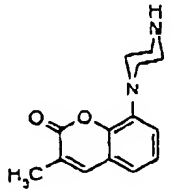

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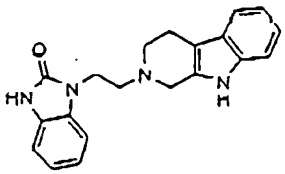

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5-HT release induced by 5-CT in guinea-pig brain slices *in vitro*. The same patent application also includes 1-NP derivatives substituted in position 7 by an aryl piperazine residue as exemplified by compound 22 which binds to human 5-HT$_{1D}$, 5-HT$_{1B}$ and 5-HT$_{1A}$ receptor subtypes with K_i values of 0.68, 0.28 and 50 nM, respectively. This compound is also reported to be a silent antagonist at 5-HT$_{1B}$ sites with no detectable intrinsic activity. These results demonstrate that the introduction of appropriate substituents in position 7 of 1-NP, which does not discriminate between 5-HT$_{1A}$, and 5-HT$_{1B/1D}$ receptors (**Table 1**), allows the design of more selective 5-HT$_{1B/1D}$ antagonists, especially *versus* 5-HT$_{1A}$ receptors. Another series of 7-substituted 1-NP derivatives have been filed in a more recent patent application by Pierre Fabre Médicament [127], including compound 23 which also demonstrated some binding selectivity in favour of 5-HT$_{1B/1D}$ receptors (K_i = 0.2 nM) compared to 5-HT$_{1A}$ (K_i = 4.3 nM).

Several other 7-substituted aminonaphthalene derivatives have been claimed by Pfizer [128,129] as 5-HT$_1$ agonists or antagonists, as illustrated by compounds 24 and 25, but their selectivity between 5-HT$_{1A}$, 5-HT$_{1C}$ and 5-HT$_{1B/1D}$ receptors has not been reported (the compounds are claimed to bind to all three receptor subtypes) and their intrinsic activity at 5-HT$_{1B/1D}$ sites has not been clearly established. However, in the latter case [129], compounds of the invention are said to antagonise the 5-HT$_{1B/1D}$ agonist-induced hypothermia in guinea-pig, thus supporting their characterisation as 5-HT$_{1B/1D}$ antagonists. The same biological properties (agonism/antagonism at 5-HT$_{1A/1B/1D}$ receptors) have also been disclosed by Pfizer [130] for 4-substituted indole derivatives illustrated by compound 26, which are claimed (as in the two other Pfizer patent applications) to be useful for treating depression, anxiety, migraine or hypertension.

Structurally close 4-(1-piperazinyl)benzo[b]thiophene derivatives (as for example compound 27) had previously been claimed by Merrell Dow Pharmaceuticals [131] as mixed 5-HT$_{1A}$ and 5-HT$_{1B/1D}$ agents. Several of these compounds have recently been reported to exhibit high affinity for both receptor subtypes with varying degrees of agonist and antagonist activity [45]. Evidence has been presented that certain of these compounds can reduce serotonin-induced contraction of canine saphenous veins and may be useful in the treatment of angina. A more recent patent application from Pierre Fabre Médicament [132] describes benzothienyl derivatives of aryl piperazines as for example compound 28 which binds to 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$ human receptor subtypes with K_i values respectively of 107, 1.5 and 3.4 nM with no intrinsic activity (as assessed by the cAMP model in CHO-K$_1$ cells) at 5-HT$_{1B}$ receptors. Such antagonists are also reported to inhibit the 5-HT induced contraction of rabbit saphenous vein and to block the inhibition of 5-HT release induced by 5-CT in guinea-pig brain slices.

It is worth noting that a group of new 1-(2H-1-benzopyran-2-one-8-yl)piperazines (for example, compound 29), has been claimed by Duphar [133] as new antidepressant and anxiolytic agents with improved activity due to their dual 5-HT$_{1A}$ receptor-agonist and 5-HT$_{1B/1D}$ receptor-antagonist effects, but no suitable biological data is given.

Finally, a recent patent application from Eli Lilly [134] describes new pyridoindole derivatives (e.g., compound 30) as serotoninergic modulators useful for treating depression among many other potential uses. Such compounds are claimed to be particularly selective for 5-HT$_{1D}$ receptor subtypes with non-negligible affinity for 5-HT$_{1B}$ and 5-HT$_{2A}$ receptors but no data concerning the 5-HT$_{1B/1D}$ antagonist properties of these compounds have been reported.

Finally, it is worth mentioning that a new endogenous cerebral tetrapeptide (LSAL) called '5-HT-moduline' has been recently identified as interacting specifically with the 5-HT1B/1D receptor subtypes [46]. Although this compound cannot be considered as an antagonist but rather as an allosteric modulator, its interaction with 5-HT1D/1B receptor corresponds to a decrease in the functional activity of the receptor (i.e., a decrease in the inhibitory effect of a 5-HT1B/1D agonist on the evoked release of 5-HT from synaptosomal preparation). It has been suggested [47] that 5-HT-moduline may play an important role in the physiological mechanism involving the serotonergic system, particularly in mechanisms corresponding to the elaboration of an appropriate response of the central nervous system (CNS) to a given stimulus. A patent application [135] from the Institute Pasteur (Paris) covers a series of peptides including LSAL as compounds able to modify serotonergic transmission with diagnostic and therapeutic applications.

Combination of 5-HT1B/1D antagonists with one or more other therapeutic agents has been covered by almost all patent applications mentioned in this review, with a particular emphasis on the combination with known antidepressants as for example tricyclic derivatives, monoamine oxidase inhibitors or SSRIs. More specifically, a patent application describing compositions comprising a carrier, a naphtyl compound and a 5-HT re-uptake inhibitor has been filed by Pfizer [136]; in these compositions, the naphtyl compound has the structural characteristics of a 5-HT1B/1D ligand (analogues of compound 24) and the preferred re-uptake inhibitor is sertraline.

Combination of 5-HT1B/D with 5-HT1A antagonists has been claimed by SmithKline Beecham [137] as a method to treat CNS disorders (including depression) likely to be much more effective than administration of a single 5-HT1B/1D or 5-HT1A antagonist. Evidence for the positive effect of the combination has been given by microdialysis studies in conscious guinea-pig *in vivo* which show that while GR-127935 and WAY 100,635 (a potent, silent 5-HT1A antagonist) are almost inactive when given alone, combination of the two derivatives leads to a 405% increase of 5-HT synaptic levels. This potentiation of GR-127935 by a 5-HT1A antagonist on 5-HT release can be compared to the well-known potentiation of SSRIs by 5-HT1A antagonists (for example, pindolol) which has been recently confirmed by clinical results in depressed patients [48]. Altogether, these combination results further support the hypothesis that 5-HT1B/1D antagonists could be useful as fast-acting antidepressants or could improve and/or accelerate the onset of antidepressant effects of SSRIs.

GR-127935 has recently been shown to potentiate the effects of paroxetine on 5-HT efflux in the rat dorsal raphe nucleus [49], and, more importantly, the combined administration of GR-127935 and sertraline synergistically increases 5-HT release in the guinea-pig hypothalamus *in vivo* [50]. These results support the notion that activation of terminal 5-HT1B/1D autoreceptors by elevated 5-HT levels reduces the net increase in extracellular 5-HT levels following acute treatment with SSRIs.

5. Expert opinion

During the last five years, important progress has been made regarding the understanding of the physiological role and the pharmacological characterisation of central 5-HT1B/1D receptors, leading to the conclusion that these receptors may play an important role in depression by controlling serotonin neurotransmission and to the working hypothesis that 5-HT1B/1D antagonists may be useful as new antidepressant drugs.

Preliminary studies with non-selective 5-HT1B/1D antagonists like 1-NP or methiothepin have been very important to establish the function of presynaptic 5-HT1B/1D autoreceptors in the control of 5-HT release and its relationship with the delay of action observed with SSRIs. Further important advances in the understanding of 5-HT1B/1D receptors function and potential therapeutic applications have been made possible thanks to GR-127935, the first selective putative 5-HT1B/1D antagonist which will certainly remain as a reference pharmacological tool despite the fact that this particular compound shows non-negligible intrinsic activity at 5-HT1B/1D sites. However, no conclusion can be reached from the studies with GR-127935 concerning the potential of silent 5-HT1B/1D antagonists as antidepressants and the disappointing results obtained so far with that particular compound in *in vivo* 5-HT release experiments cannot be considered as conclusive.

Only recently have examples of compounds which appear to correspond to selective, silent 5-HT1B antagonists (or inverse agonists) been reported. These include GR-55562 and SB-224289 which have been characterised respectively as a silent antagonist and an inverse agonist at human cloned 5-HT1B receptors. Further pharmacological characterisations of these compounds (especially their expected ability to increase synaptic serotonin release) will be of primary importance to evaluate the potential of 5-HT1B antagonists (or inverse agonists) for the treatment of depression. The recent patent literature summarised in this review suggests that other new 5-HT1B/1D receptor silent antagonists will also emerge rapidly and will

probably offer new pharmacological tools or/and drug candidates for the future. Such compounds will be particularly useful to assess further the functional role of 5-HT1B/1D receptors and will certainly help answering a few important questions:

- What presynaptic terminal auto-receptor subtype(s) are involved in controlling 5-HT release? To date, all data support the hypothesis that the 5-HT1B receptor subtype is the main autoreceptor involved at the terminal level. However, so far, no functional role has been identified for central 5-HT1D receptors and it cannot be excluded that presynaptic 5-HT1E or 5-HT1F receptors may also be implicated in the control of 5-HT release.

- Will a pure, selective, silent and potent 5-HT1B/1D antagonist be able to increase 5-HT release to an extent which is sufficient to have antidepressant efficacy? The extensive pharmacological work done with GR-127935 failed to answer this question, probably because the compound is not a silent antagonist. The comparison of 5-HT1B/1D silent antagonists with inverse agonists will be very informative on that point of view. The concept of an inverse agonist may open a fascinating field of investigation on the control of 5-HT release by 5-HT1B/1D receptors. As discussed previously [21], it may be more appropriate to develop inverse agonists than neutral (or silent) antagonists for depression, since the latter ones would only operate in the presence of a sufficient 5-HT tone. In the absence of 5-HT (or when synaptic 5-HT levels are low, as hypothesised for depressive states), an inverse agonist may still be able to increase 5-HT release, whereas an antagonist will probably be only weakly active.

- Another subsequent question arising from the relative importance of 5-HT1B/1D autoreceptors in controlling 5-HT release is concerned with the ability of 5-HT1B/1D antagonists to be effective alone; in other words, will these compounds require combination with SSRIs to express antidepressant activity? Data available today support the hypothesis that 5-HT1B/1D antagonists potentiate (and perhaps accelerate the onset of) the antidepressant effects of SSRIs.
  Moreover, other mechanisms, for example the control of somatodendritic 5-HT1A receptors, have also been recently identified as important in the control of 5-HT release mediated by SSRIs. Thus, molecules combining 5-HT1D antagonism with 5-HT1A properties will be interesting to compare with pure, selective 5-HT1B/1D ligands.

- Besides 5-HT1B/1D presynaptic terminal autoreceptors, which are the targets for antagonists in depression, 5-HT1B/1D receptors also exist as postsynaptic receptors (perhaps involved in thermoregulation, locomotion, feeding and sexual behaviours), presynaptic heteroreceptors (controlling release of neurotransmitters other than 5-HT), somatodendritic receptors (perhaps modulating 5-HT neuronal firing rate) and peripheral vascular receptors (implicated in vasoconstriction). Thus, the consequences of antagonising these receptors will have to be studied carefully. To date, few data have been reported concerning the physiological or behavioural consequences of inhibiting 5-HT1B/1D receptors. Furthermore, the cardiovascular safety of antagonists remains to be established.

In addition to evidence suggesting that 5-HT1B/1D antagonists might be effective as antidepressant agents, it may be speculated that other therapeutic indications will be investigated in the future, including angina, hypertension, OCD, anxiety, panic attacks, mood disorders, eating disorders, neurodegenerative diseases or disorders of the gastrointestinal tract. Recent investigations concerning the growth-stimulatory effects of serotonin on carcinoid cells, the possible involvement of 5-HT1B/1D receptors in this effect [51,52] and recent data demonstrating that stimulation of cloned human 5-HT1B receptors in C6 glial cells can promote cell growth [53] together suggest that 5-HT1B/1D antagonists may find utility as antitumour agents.

In conclusion, the extensive search for 5-HT1B/1D antagonists or inverse agonists has been illustrated during the last three years by important patent activity and the emergence of new, potent, and selective molecular entities. The time is now coming for their pharmacological evaluation in biochemical or behavioural models of depression. It can be expected that the first clinical trials will take place in the near future and that these will undoubtedly provide data needed to realise the potential of 5-HT1B/1D antagonists or inverse agonists in the treatment of depression and other disorders.

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CHAPTER 12

Regulation of 5-HT Release in the CNS by Presynaptic 5-HT Autoreceptors and by 5-HT Heteroreceptors

M. Göthert and E. Schlicker

A. Introduction: Definitions and Scope

The amount of serotonin (5-hydroxytryptamine; 5-HT) released from the varicosities of the serotoninergic axon terminals in response to invading action potentials at a given frequency is by no means constant. As generally accepted now, exocytotic release of 5-HT can be significantly modified by presynaptic receptors, i.e. receptors located on the serotoninergic axon terminals (for reviews, see Moret 1985; Middlemiss 1988; Starke et al. 1989; Göthert 1990). When such receptors are stimulated by 5-HT released from the serotoninergic axon terminals, they are termed presynaptic 5-HT autoreceptors. When receptors on the serotoninergic axon terminals are activated by other transmitters released from non-serotoninergic neurons, they are denoted as presynaptic heteroreceptors. In the latter case, the axon terminals of these neurons may form axo-axonic synapses with the serotoninergic terminals, or the interacting neighbouring neurons may release their transmitter non-synaptically into the extracellular space and, thus, may influence larger neuronal territories including the serotoninergic axon terminals (Beaudet and Descarries 1978, Törk 1990).

The present report will focus mainly on inhibitory presynaptic 5-HT autoreceptors. Such receptors, via an ultrashort inhibitory feedback loop, probably play an important physiological role in the fine regulation of 5-HT release. The presynaptic 5-HT autoreceptors have to be distinguished from 5-HT autoreceptors on the cell bodies and dendrites of the serotoninergic neurons, i.e. the somadendritic 5-HT autoreceptors (Sprouse and Aghajanian 1986, 1987; Hjorth and Magnusson 1988; Schechter et al. 1990; Pineyro et al. 1995a). The latter can be activated by 5-HT released from the somadendritic area or recurrent branches of the serotoninergic axon of the same neuron or from axons of other serotoninergic neurons innervating the cell bodies and dendrites of the neuron under consideration. According to the location of these receptors, their stimulation induces a decrease in neuronal cell firing (5-HT_{1A} receptors; Sprouse and Aghajanian 1986, 1987; Hjorth and Magnusson 1988; Schechter et al. 1990) or in somadendritic 5-HT release (5-HT_{1D} receptors; Pineyro et al. 1995a). In this report, these receptors, which certainly are also of high functional importance, will not be reviewed in detail, but, when

relevant, will be considered in comparison with the presynaptic 5-HT autoreceptors.

Whereas the cell bodies and dendrites of the serotoninergic neurons are located in a limited brain area, namely the raphe nuclei of the brainstem, their axons project into virtually all parts of the CNS (Törk 1990; Chap. 2, this volume) and, accordingly, presynaptic 5-HT autoreceptors are widely distributed within the brain and spinal cord (see, e.g. Starke et al. 1989). It may be deduced from the presynaptic location of heteroreceptors on the serotoninergic axon terminals (e.g. presynaptic α_2-heteroreceptors; Göthert and Hurt 1980; Frankhuyzen and Mulder 1980; Göthert et al. 1981) that signals can directly be conducted from other brain areas via non-serotoninergic nerves (e.g. from the locus coeruleus via noradrenergic neurons) to the serotoninergic varicosities. At these sites the transmitter released shares the property of 5-HT itself to modify the strength of the chemical signal (i.e. the amount of 5-HT available in the synaptic cleft) at the location where this signal is formed. In other words, such a modulatory-type action of non-serotoninergic transmitters on the release of serotonin exerted via presynaptic heteroreceptors resembles the restraining effect of extracellular 5-HT acting on its autoreceptors.

The presynaptic heteroreceptors on the serotoninergic nerves will not be considered here in detail, since their occurrence and function has recently been reviewed (Göthert and Schlicker 1991). However, presynaptic heteroreptors may also come into play in the context of the main topic of the present report, namely the autoregulation of 5-HT release. This process may not only be operative via 5-HT autoreceptors, but may also involve neuronal circuits with at least one interneuron. In the case of *one* interneuron only, it should be activated by 5-HT via a 5-HT receptor, resulting in the release of a transmitter which acts via its heteroreceptor on the serotoninergic axon terminals. If *several* neurons are involved in such a feedback loop, the first one would be endowed with the 5-HT receptor; the last one would release the transmitter activating its presynaptic heteroreceptor on the serotoninergic terminal. Activation of such a feedback loop may result in a facilitation or inhibition of 5-HT release and, thus, may mimic the function of a facilitatory or inhibitory presynaptic 5-HT autoreceptor, respectively. Whereas the occurrence of an inhibitory neuronal circuit consisting of several neurons will only briefly be considered in Sect. D.I.4, the probable involvement of such a feedback loop will be more extensively discussed in the context of, e.g. 5-HT receptors mediating facilitation of 5-HT release (Galzin et al. 1990; Galzin and Langer 1991; Blier and Bouchard 1993).

5-HT receptors are also present as presynaptic 5-HT heteroreceptors on non-serotoninergic axon terminals (see, e.g. Göthert 1990; Cassel and Jeltsch 1995). These receptors, which are, as a rule, less well investigated (except 5-HT heteroreceptors on cholinergic axon terminals; Cassel and Jeltsch 1995), will not be dealt with in this report.

B. Methodological Approaches

The operation of 5-HT receptors mediating inhibition or facilitation of 5-HT release has mainly been proved by experiments in which the effects of 5-HT receptor ligands on the release of 5-HT were investigated either in vitro or in vivo. According to the concept underlying such investigations, a receptor agonist should inhibit or increase 5-HT release in a manner susceptible to blockade by an appropriate 5-HT receptor antagonist. If the receptor is tonically activated by 5-HT, the antagonist, given alone, should produce an effect opposite to that of the agonist. Such functional studies cannot only prove the existence of presynaptic 5-HT receptors, but also provide valuable information about their pharmacological properties, function, potential physiological role and, by application of appropriate pharmacological tools, the site and mechanism by which they influence neurotransmitter release.

1. In Vitro Studies

Most investigations available have been carried out with slices or synaptosomes. As a rule, the preparations were preincubated with radioactively labelled neurotransmitter, and the stimulation-evoked overflow of radioactivity or (after column chromatography) labelled 5-HT from the superfused (in a few cases, incubated) preparations has been determined (for review, see, e.g. MIDDLEMISS 1988; STARKE et al. 1989). In some investigations, slices were incubated with a radioactive precursor leading to the synthesis of labelled 5-HT, whose overflow in response to stimulation was studied (HAMON et al. 1974). It is generally accepted that stimulation-evoked overflow of tritium or labelled 5-HT, in particular in the presence of an inhibitor of neuronal 5-HT uptake, reflects the release of endogenous 5-HT. Therefore, in these cases, the term "5-HT release" will be used throughout the subsequent sections. Very few experiments were based on the investigation of the stimulation-evoked overflow of endogenous 5-HT; as a rule, the latter has been determined by means of fast cyclic voltammetry. This technique has been applied in slices of the rat dorsal raphe nucleus (STARKEY and SKINGLE 1994; DAVIDSON and STAMFORD 1995). In most investigations, either electrical field stimulation or high K^+ was used to depolarize the cell membrane of the respective neurons and, thus, to stimulate transmitter release. Recently, *N*-methyl-D-aspartate (NMDA) has also been applied for this purpose (FINK et al. 1996).

The advantage of the use of superfused synaptosomes, consisting mainly of isolated varicosities, for such experiments is that the data obtained provide evidence for the presynaptic location of the receptors involved (CERRITO and RAITERI 1979). Furthermore, such synaptosomal experiments make it possible to study the function of presynaptic receptors undisturbed of the influence of its endogenous ligand. In conventional experiments on slices, the endogenous agonist must be assumed to be present in the biophase of the presynaptic

receptor under consideration at sufficiently high concentration to interact with an additional exogenous agonist. However, the interaction of the endogenous neurotransmitter can also be eliminated in experiments in which the overflow of radioactivity or a labelled transmitter is elicited by a small number of electrical impulses (as a rule up to four) at high frequency (100 Hz). In such "pseudo-one-pulse" ("POP") experiments, the concentration of the endogenous transmitter in the biophase of the corresponding presynaptic receptor is too low to stimulate it, and, on the other hand, the radioactive signal is strong enough to be measurable (Singer 1988).

II. In Vivo Studies

Intracerebral microdialysis has been the most frequently applied technique in anaesthetized or freely moving animals. 5-HT and its metabolites in the dialysate have been measured by means of high-pressure liquid chromatography (HPLC) (Brazell et al. 1985; Sharp et al. 1989). This technique makes it possible to directly apply 5-HT receptor agonists and/or antagonists to the brain area under investigation and to simultaneously determine their influence on the overflow of 5-HT and its metabolites. The effect of direct application can be compared to that of systemic administration of the drugs (Assie and Koek 1996). This comparison provides hints at whether or not the drug passes the blood-brain barrier, and allows the determination of the overall importance of the presynaptic 5-HT autoreceptor in the control of 5-HT release. In studies in which the drugs are exclusively injected systemically, it is difficult to decide whether an effect observed is due to a direct action at the serotoninergic nerve endings or whether the drugs primarily act in the somadendritic area of this neuron. A further possibility is that the drugs may influence the serotoninergic neuron indirectly by acting at non-serotoninergic nerves endowed with 5-HT receptors of a subtype identical to, or different from, the presynaptic 5-HT autoreceptors (see Sect. C.II, D.II).

In some of the in vivo studies, fast cyclic voltammetry has been used to determine 5-HT release (Marsden et al. 1986; Pineyro et al. 1995a). The disadvantage of this technique is the lower specificity compared to HPLC, the main advantage being the high topographic resolution, which makes it possible to measure release in very small brain areas.

Finally, electrophysiological methods have been successfully applied to study presynaptic 5-HT autoreceptor function in vivo (Chaput et al. 1986; Chaput and De Montigny 1988). This approach is based on a comparison of the depressant response of hippocampal pyramidal neurons to directly applied exogenous 5-HT and of endogenous 5-HT, released in response to electrical stimulation of the ascending serotoninergic axons. Application of a 5-HT receptor antagonist which leaves the postsynaptic pyramidal 5-HT receptor at least largely unaffected but blocks the presynaptic 5-HT autoreceptors electively enhances the depressant response to stimulation of the afferent

serotoninergic neurons without influencing the response to directly applied 5-HT.

C. Identification, Location and Physiological Role of 5-HT Receptors Mediating Inhibition or Facilitation of 5-HT Release

Up to 1990 presynaptic 5-HT autoreceptors were reviewed a number of times, either specifically (Moret 1985; Middlemiss 1988) or in a more general context of various presynaptic receptors (Göthert 1982; Timmermans and Thoolen 1987; Starke et al. 1989; Göthert 1990). In order to avoid redundance, the present article will focus mainly on recent developments not yet covered in the review by Starke et al. (1989) coauthored by one of us. However, key findings and important conclusions relevant to presynaptic 5-HT autoreceptors will be briefly summarized here, irrespective of whether or not they have already been extensively reviewed.

1. Inhibitory Presynaptic 5-HT Autoreceptors

1. Identification and Location

Evidence for the existence of inhibitory presynaptic 5-HT autoreceptors was presented first by Farnebo and Hamberger (1971). These authors showed that the non-selective 5-HT receptor agonist, LSD, inhibited 5-HT release in rat brain cortex slices, a finding which was confirmed by Hamon et al. (1974). Farnebo and Hamberger (1974) demonstrated that the effect of LSD was shared by other 5-HT receptor agonists and that the non-selective 5-HT receptor antagonist methiothepin increased 5-HT release. On the basis of these findings, the concept of an inhibitory feedback regulation of 5-HT release was developed which was supported by Cerrito and Raiteri (1979) using rat hypothalamic synaptosomes and by Göthert and Weinheimer (1979) using rat brain cortex slices. These studies revealed that in the presence of an inhibitor of the neuronal 5-HT transporter, 5-HT itself when applied exogenously inhibited 5-HT release and that the effect of 5-HT was counteracted by methiothepin; furthermore, the results of the experiments on cortical slices confirmed those by Farnebo and Hamberger (1974) in that methiothepin, given alone, disinhibited, i.e. increased, 5-HT release.

In the following years, inhibitory 5-HT autoreceptors were also identified by means of basically identical techniques in other regions of the rat brain and in the brain of other species, including mouse, guinea pig, rabbit, pig and man (Fig. 1; Table 1). Generally speaking, presynaptic 5-HT autoreceptors have been identified in every region of the mammalian CNS which has been investigated for this purpose (Table 1). During the last 8 years, the occurrence and operation of these receptors in the brain of the species mentioned so far have been repeatedly confirmed (Wichman et al. 1989; Feuerstein et al. 1992;

Table 1. Occurrence of inhibitory 5-HT autoreceptors in various brain regions of the rat and other species. P, presynaptic location has been proved; E, indirect evidence for activation by endogenous 5-HT has been presented; C, the receptor has been classified according to its pharmacological properties in terms of 5-HT_{1B} and "5-HT_{1D}". More recent findings not yet considered in the review by Starke et al. (1989; including brain regions and species in which autoreceptors had not yet been identified) are underlined. References for autoreceptors characterized or classified during the last 8 years: cortex of guinea pig: Hoyer and Middlemiss (1989), Limberger et al. (1991), Bühlen et al. (1996a); cortex of rabbit: Limberger et al. (1991); cortex of mouse and rhesus monkey: Schipper (1990); cortex and hypothalamus of mouse: Schlicker, Fink and Göthert (unpublished); hippocampus of guinea pig: Wilkinson and Middlemiss (1992); periaqueductal gray of the rat: Versteeg et al. (1991); superior colliculus of the rabbit: Wichmann et al. (1989); hypothalamus and substantia nigra of the guinea pig: Moret and Briley (1995); corpus striatum (caudate nucleus) of rabbit: Feuerstein et al. (1992); human hippocampus: Schlicker et al. (1996)

Cerebral cortex		Hypothalamus	
Rat	P, E, C	Rat	P, E, C
Mouse	E, C	Mouse	E
Guinea pig	P, E, C	Guinea pig	E
Rabbit	E, C	Rabbit	E
Pig	P, E, C		
Rhesus monkey	C	Superior colliculus	
Man	P, E, C	Rabbit	E
		Periaqueductal gray	
Hippocampus		Rat	
Rat	P, E, C		
Guinea pig	E	Cerebellum	
Rabbit	E, C	Rat	P, C
Man	E	Mouse	E
Nucleus accumbens		Medulla oblongata	
Rat	P	Rat	E
Corpus striatum		Spinal cord	
Rat		Rat	P, E, C
Rabbit	E, C		
Substantia nigra			
Guinea pig	C		

Wilkinson and Middlemiss 1992; Moret and Briley 1995; Bühlen et al. 1996a; Fink et al. 1996) and have, in addition, been demonstrated in the cerebral cortex of another species, namely the rhesus monkey (Schipper 1990).

In view of the potential role of brain serotoninergic neurotransmission in the pathogenesis and drug therapy of neuropsychiatric diseases (see Sect. G and, e.g. Göthert 1991; Peroutka 1991; Chopin et al. 1994; Chap. 10, this volume), it was of particular interest to investigate whether presynaptic 5-HT autoreceptors also occur in the human hippocampus. This has recently been proven in experiments on human hippocampal slices (Schlicker et al. 1996).

The occurrence of inhibitory 5-HT autoreceptors has been shown not only in the in vitro experiments discussed so far, but also in vivo in the brain of anaesthetized and freely moving animals. As summarized in Table 2, 5-HT

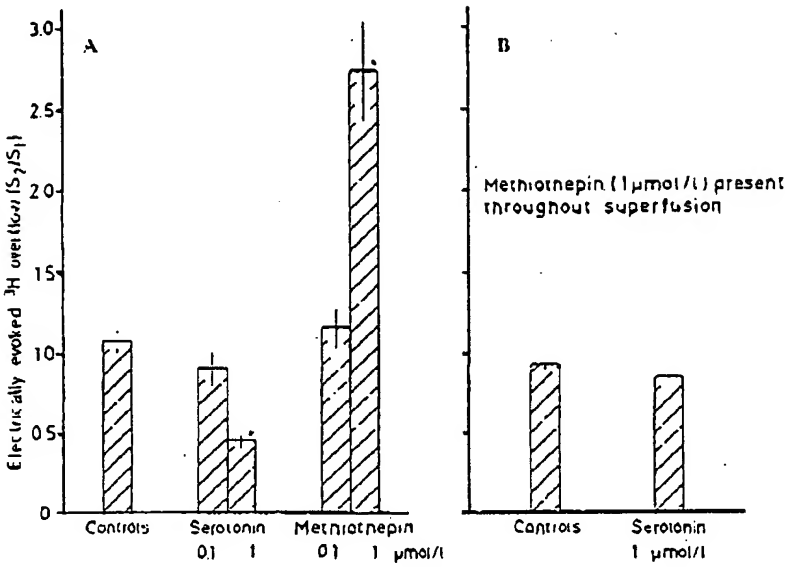

Fig. 1A,B. Inhibition by unlabelled serotonin (5-HT) and facilitation by methiothepin of the electrically (3 Hz) evoked overflow of tritium (consisting of more than 80% [³H]5-HT) from superfused human cerebral cortical slices (**A**) and abolition of the inhibitory effect of 5-HT by methiothepin (**B**). The slices were preincubated with [³H]5-HT and superfused in the presence of the 5-HT uptake inhibitor, paroxetine (3.2 *μM*). Two periods of stimulation were applied after 40 and 90 min of superfusion (S_1, S_2) and the ratios of the overflow evoked by S_2 over that evoked by S_1 (S_2/S_1) are given (means + SEM; $n = 4–7$; $^*P < 0.001$). **A** 5-HT or methiothepin was present from the 65th min of superfusion onward; **B** methiothepin was present throughout superfusion and 5-HT from the 65th min of superfusion onward. (Modified from SCHLICKER et al. 1985)

receptor agonists inhibited 5-HT release in a manner sensitive to blockade by methiothepin, which given alone facilitated 5-HT release. In these studies the technique of in vivo microdialysis was used and 5-HT release was quantified by HPLC. Table 2 exclusively contains data from studies in which the drugs were administered directly via the dialysis probe.

Investigations of 5-HT release in the raphe nuclei were not included since in this region it is difficult to distinguish unequivocally between responses mediated by presynaptic autoreceptors on recurrent axon terminals, by somadendritic autoreceptors or by both (STARKEY and SKINGLE 1994; DAVIDSON and STAMFORD 1995). Exclusion of 5-HT receptors modulating 5-HT release in the raphe nuclei from a review on *presynaptic* 5-HT autoreceptors is the more justified as results obtained in this brain area of the rat with subtype-selective 5-HT receptor agonists and antagonists strongly support the view that *somadendritic* 5-HT autoreceptors modulate 5-HT release from the cell bodies and dendrites (PINEYRO et al. 1995a). These somadendritic 5-HT autoreceptors belong to the r5-HT_{1D} subtype (PINEYRO et

MINNES SÄNDNINGS RAPPORT

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FIL NUMMER : 870 *** MISSLYCKAD SÄNDN.LISTA ***

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August 23, 1999

Our ref.
J 1833-1 TW

Your ref.
54092-YCT

BY FAX – 68 PAGES - ORIGINAL BY MAIL

Re: Taiwanese Patent Application No. 87111888

Dear Sirs,

We refer to your letter of 19 July 1999 reporting a preliminary notice of final rejection and your comments thereon.

Below please find our comment thereon and instructions to a response thereto. The numbers below refer to your numbers used in your comments.

(1) Please add into the abstract the definitions of R_6, R_7 and R_8 as set forth in Claim 1.

(2) Enclosed please find claims 1, 34 and 36 wherein we have underlined all substituents for which we have technical support in the examples. Please study our underlinde and prepare arguments based thereon which are reasonable according to Taiwanese patent practice.

(3) As regards Claims 16-18, we accept your suggestion under 3(b) of your comments.

Furthermore, please amalgamate Claim 19 with Claim 20.

There is an intimate correlation between the h5-HT_{1B} receptor and the indications recited in the claims. This is for example known from the enclosed two publications "Regulation of 5-HT Release in the CNS by Presynaptic 5-HT Autoreceptors and by 5-HT Heteroreceptors", M. Göthert et al. pp. 307-360, and "5-$HT_{1B/1D}$ antagonists and depression", S. Halazy et al. pp. 339-352.

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Table 2. Effects of 5-HT receptor agonists and antagonists on 5-HT release in the rat and guinea pig brain in vivo. 5-HT release was determined by the microdialysis technique. 5-HT receptor ligands and 5-HT uptake blockers were administered locally through the dialysis probe (the concentration obtained in the brain tissue is about 20-fold lower). Note that the 5-HT receptor ligands indicated have not been examined in each of the studies

Species, CNS region	References (anaesthetized or freely moving animal? 5-HT uptake blocker present?)	5-HT receptor ligands (μmol/l) causing				Comment
		Inhibition		Facilitation		
Rat, cerebral cortex	Auerbach and Hjorth (1995; CH-anaesthetized; citalopram)	RU 24969 CP-93, 129	0.1–10 10			
Rat, hippocampus	Claustre et al. (1991; CH-anaesthetized) Hjorth and Tao (1991; CH-anaesthetized; citalopram) Martin et al. (1992; CH-anaesthetized; citalopram) Hjorth and Sharp (1993; CH-anaesthetized; citalopram) Auerbach and Hjorth (1995; CH-anaesthetized; citalopram) Bosker et al. (1995; freely moving; fluvoxamine)	RU 24969 TFMPP CP-93, 129	0.1–10 10 3–10	Methiothepin (–)-Penbutolol	10 1	No effect of 8-OH-DPAT (up to 100 μmol/l); effect of RU 24969 and CP-93, 129 antagonized by methiothepin and/or (–)-penbutolol; data are compatible with a 5-HT_{1B} receptor[a]

Rat, diencephalon	Auerbach et al. (1991; freely moving, fluoxetine)	RU 24969	1			No effect of TFMPP 10 μmol/l
Guinea pig, cerebral cortex	Sleight et al. (1990; CH-anaesthetized) Claustre et al. (1991; CH-anaesthetized) Lawrence and Marsden (1992; freely moving) Hutson et al. (1995; isoflurane-anaesthetized) Skingle et al. (1995; CH-anaesthetized)	5-CT Oxymetazoline Sumatriptan	0.1–10 10 0.01–10	Methiothepin	33–100	No effect of RU 24969; facilitatory effect of GR 127935 0.1 μmol/l in the study by Skingle et al. (1995) but no effect of the same drug (10–100 μmol/l) in the study by Hutson et al. (1995)
Guinea pig, substantia nigra	Briley and Moret (1993; freely moving) Moret and Briley (1995; freely moving)			Methiothepin 1-(1-Naphthyl)-piperazine	100 1–100	

'Suggested both by the effectiveness of the selective 5-$HT_{1A/B}$ receptor agonist CP-93, 129 and the lack of effect of 8-OH-DPAT (a 5-HT_{1A} receptor agonist with moderate affinity for 5-HT_{1B} and virtually no affinity for 5-HT_{1B} receptors)
CH, chloral hydrate; TFMPP, m-trifluoromethy phenylpiperazine; 5-CT, 5-carboxamidotryptamine; 8-OH-DPAT, 8-hydroxy-2(di-*n*-propylamino)tetralin; RU 24969, 5 methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole.

al. 1995a; new nomenclature of 5-$HT_{1B/1D}$ receptors; see Sect. D.1.3). Via these receptors, release can be controlled independently of firing activity and the firing-regulating 5-HT_{1A} autoreceptors (Starkey and Skingle 1994; Pineyro et al. 1996). 5-HT_{1D} autoreceptors also modulate 5-HT release in the mesencephalic raphe of guinea pigs (El Mansari and Blier 1996); it may be assumed that these receptors are also located in the somadendritic area of the serotoninergic neurons.

As already mentioned above (Sect. B), the presynaptic location of receptors can be proved by experiments in synaptosomes. Another approach consists of the functional isolation of the axon terminals in brain slices by interrupting the impulse traffic along the axons by tetrodotoxin. Under this condition K^+-evoked 5-HT release cannot be influenced by short neuronal circuits within the slices. However, a theoretical, but very improbable, possibility which is not excluded by such experiments is that 5-HT, via a presynaptic 5-HT heteroreceptor, primarily stimulates the release of a neurotransmitter from a non-serotoninergic axon terminal. This, in turn, modifies 5-HT release via a presynaptic heteroreceptor for its transmitter. Both slices superfused in the presence of tetrodotoxin and synaptosomes have been used to provide evidence for the presynaptic location of inhibitory 5-HT autoreceptors in several areas of the rat CNS (Starke et al. 1989). In Table 1, the areas in which the presynaptic location of the inhibitory autoreceptors has been proved are marked by "P".

Recently, the presynaptic location of inhibitory 5-HT autoreceptors has also been demonstrated in the human (Maura et al. 1993; Fink et al. 1995; Fig. 2) and guinea pig cerebral cortex (Bühlen et al. 1996a) by means of experiments on synaptosomes. In this context, it should be noted that the guinea pig brain represents an important model for the development of ligands of presynaptic 5-HT autoreceptors, which may become useful for the treatment of human neuropsychiatric diseases (Chopin et al. 1994; Chap. 10, this volume). Furthermore, in experiments on slices superfused with solution containing tetrodotoxin, the presynaptic location of the inhibitory 5-HT autoreceptors in the human hippocampus was made probable, since under this condition 5-carboxamidotryptamine 5-CT still inhibited the K^+-evoked 5-HT release in a manner sensitive to antagonism by methiothepin (Schlicker et al. 1996).

As in previous studies (for review see Starke et al. 1989), attempts have been made in rats to more directly prove the location of the presynaptic 5-HT autoreceptors which belong to the 5-HT_{1B} subtype (see Sect. D.1) on the serotoninergic axon terminals themselves. In these experiments, the density of 5-HT_{1B}-binding sites has been determined after chemical axotomy with 5,7-dihydroxytryptamine. Either no change or even an increase in 5-HT_{1B} receptor density occurred (Frankfurt et al. 1993, 1994), suggesting that the presynaptic 5-HT_{1B} autoreceptors do not account for a major proportion of the total number of these receptors and that 5-HT_{1B} receptors which are not located on 5-HT neurons appear to be upregulated. Comparison of the levels of 5-HT_{1B} receptor messenger RNA with the densities of 5-HT_{1B}-binding sites in mouse

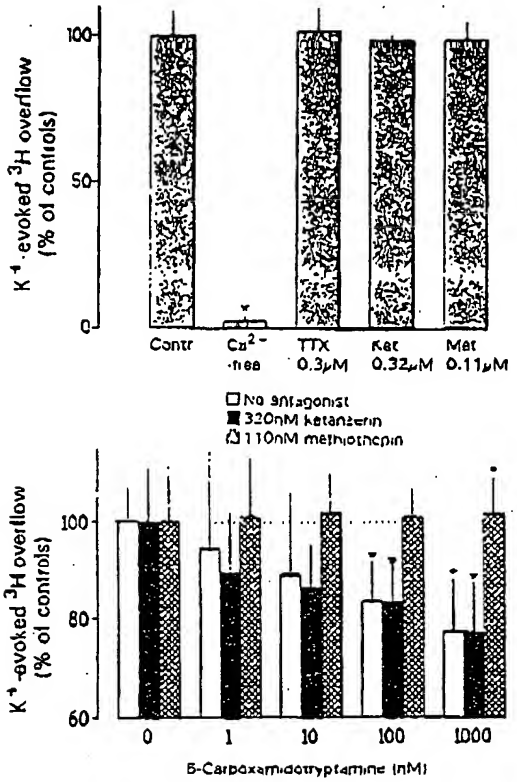


Fig. 2. Effects of serotonin (5-HT) receptor ligands on the K^+ (25 m*M*)-evoked tritium overflow from superfused human cerebral cortical synaptosomes preincubated by [^{3}H]5-HT. *Upper panel*, lack of influence of ketanserin (*Ket*), methiothepin (*Met*) or tetrodotoxin (*TTX*) on the evoked tritium overflow and abolition of overflow by omission of Ca^{2+} from the superfusion fluid; *Contr*, drug-free controls. *Lower panel*, inhibition by 5-carboxamidotryptamine (5-CT) of evoked tritium overflow and interaction with 5-HT receptor antagonists' effect of 5-CT not changed by Ket, but abolished by Met. Ket 320 n*M* corresponds to 4.4 times its K_i at cloned h5-HT_{1D} receptors and Met 110 n*M* corresponds to 4.4 times its K_i at cloned h5-HT_{1A} receptors (K_i values from WEINSHANK et al. 1991). The synaptosomes were superfused in the presence of the 5-HT uptake inhibitor fluvoxamine (10 μ*M*). Ca^{2+} was omitted from, and the drugs were added to, the superfusion fluid from the 20th min before stimulation onward. Means ± SEM of 6–14 experiments. $^*P < 0.05$, compared to the corresponding controls without 5-CT. $^+P < 0.05$, compared to the corresponding effect of 5-CT in the absence of Met (Modified from FINK et al. 1995)

brain areas containing cell bodies and in areas to which they project revealed that the 5-HT_{1B} receptor is localized predominantly on axon terminals (BOSCHERT et al. 1994); although this study referred to presynaptic 5-HT_{1B} heteroreceptors, the data were compatible with the above suggestion, namely

that 5-HT_{1B} autoreceptors account for only a minor fraction of the whole population of 5-HT_{1B}-binding sites.

2. Physiological Role

Do the inhibitory presynaptic 5-HT autoreceptors play a physiological role or, in other words, are they activated by endogenous 5-HT? The usual way to answer this question is to examine the effect of an appropriate 5-HT receptor antagonist on 5-HT release. If endogenous 5-HT is present in the biophase of such an inhibitory 5-HT receptor at a sufficient amount to produce a tonic activation, addition of an antagonist should modify 5-HT release in a manner opposite to that of 5-HT itself, i.e. it should facilitate 5-HT release. This approach is, however, not applicable to superfused synaptosomes, in which endogenous 5-HT is effectively removed from the biophase of the receptors by the superfusion stream. The procedure can also not be used in slices in which 5-HT release is evoked by a single electrical pulse or by the pseudo-one-pulse stimulation (few pulses administered at a very high frequency; SINGER 1988; see Sect. B.1) since in this case the amount of 5-HT released is too low to tonically activate the 5-HT autoreceptor.

It has already been mentioned previously that, in experiments on slices, the 5-HT receptor antagonist methiothepin (0.1–1 μM) given alone increased 5-HT release in various regions of the CNS of several species. These findings suggest that the presynaptic 5-HT receptors are tonically activated at the physiological frequency of electrical stimulation applied in the numerous studies (STARKE et al. 1989). In Table 1, the areas in which this has been demonstrated and, hence, a physiological role has been proved are marked by "E". In some of these areas of several species, tonic activation of the inhibitory presynaptic 5-HT autoreceptors has been found only recently. This holds true for rat spinal cord (YANG et al. 1994), mouse cerebral cortex and hypothalamus (SCHLICKER, FINK and GÖTHERT, unpublished), guinea pig cerebral cortex (WILKINSON et al. 1993), hippocampus, hypothalamus and substantia nigra (WILKINSON and MIDDLEMISS 1992; CHOPIN et al. 1994), rabbit colliculus superior and caudate nucleus (WICHMANN et al. 1989; FEUERSTEIN et al. 1992) and human hippocampus (SCHLICKER et al. 1996). Taken together it may be stated that methiothepin facilitated 5-HT release in any region of the CNS in which it has been studied. Such an effect has also been observed in in vivo experiments in which methiothepin was administered to the rat hippocampus or guinea pig cerebral cortex or substantia nigra via the dialysis probe (Table 2). These data support the view that the inhibitory presynaptic 5-HT autoreceptors play a physiological role under in vivo conditions.

II. 5-HT Receptors Mediating Facilitation of 5-HT Release

1. Identification and Location

A facilitatory feedback loop in which 5-HT receptors are involved has been identified only recently. Using superfused slices from the rat hypothalamus

and guinea pig cerebral cortex. GALZIN and LANGER (1991) showed that the 5-HT_1 receptor agonist 2-methyl-5-HT facilitated the electrically evoked 5-HT release. This effect was counteracted by the 5-HT_3 receptor antagonists ondansetron and tropisetron. Basically the same results were obtained in guinea pig hypothalamic slices (BLIER and BOUCHARD 1992). In a more detailed study by BLIER and BOUCHARD (1993) on slices of the cerebral cortex, hypothalamus and hippocampus of guinea pigs, the facilitation of the electrically (1, 3 or 5 Hz) evoked 5-HT release by 2-methyl-5-HT was confirmed, as was the sensitivity of this effect to blockade by various 5-HT_3 receptor antagonists (among others, ondansetron, tropisetron and *S*-zacopride). The enhancing effect of 2-methyl-5-HT in guinea pig hypothalamic slices was the more pronounced, the higher the frequency of stimulation with a constant number of pulses (3 Hz compared with 1 Hz) and the shorter the duration of stimulation at a constant frequency (BLIER and BOUCHARD 1993). In guinea pig hypothalamic slices, 2-methyl-5-HT also increased the K^+ (15–35 m*M*)-evoked 5-HT release (BLIER et al. 1993). The facilitatory effect of 2-methyl-5-HT on the 5-HT release evoked by 25 m*M* K^+ was antagonized by ondansetron (BLIER et al. 1993). At high concentrations, 2-methyl-5-HT even produced direct stimulation of 5-HT release from guinea pig hypothalamic slices, which was sensitive to blockade by *S*-zacopride (BLIER and BOUCHARD 1993).

A facilitatory effect of 2-methyl-5-HT on the electrically evoked 5-HT release was observed in rat frontal cortical and hippocampal slices as well (HADDJERI and BLIER 1995); this effect was blocked by the 5-HT_3 receptor antagonists BRL 46470A [(endo-*N*-(8-methyl-8-azabicyclo[3.2.1.]oct-3-yl)2,3-dihydro-3,3-dimethylindole-1-carboxamide; ricasetion] and *S*-zacopride.

The receptors involved in these effects are also operative in vivo (MARTIN et al. 1992). In the ventral hippocampus of rats anaesthetized with chloral hydrate, administration of 2-methyl-5-HT via the microdialysis probe increased dialysate 5-HT levels in a concentration-related manner. This effect was counteracted by the 5-HT_3 receptor antagonist MDL 72222 (endo-8-methyl-8-azabicyclo[3.2.1.]oct-3-yl-3,5-dichlorobenzoate).

The receptors mediating facilitation of 5-HT release are not autoreceptors (BLIER et al. 1993) but are probably located on an interneuron. This conclusion can be drawn from data of BLIER et al. (1993), who found in guinea pig hypothalamic synaptosomes that 2-methyl-5-HT did not facilitate the K^+-evoked 5-HT release, nor did it stimulate 5-HT release by itself. In agreement with this, no enhancement of the K^+-evoked 5-HT release by 2-methyl-5-HT was observed in hypothalamic slices, when tetrodotoxin was present in the superfusion fluid (BLIER et al. 1993). Hence, a neuronal circuit comprising one or more interneurons and an unknown presynaptic heteroreceptor on the seroroninergic axon terminals should be involved (see Sect. A).

2. Physiological Role

Relatively little information is so far available with respect to the potential tonic seroroninergic input to the facilitatory 5-HT receptors. In this case, 5-

HT_1 receptor antagonists should cause an inhibition of 5-HT release, but BLIER and BOUCHARD (1993) found in guinea pig hypothalamic slices that, as a rule (e.g. with ondansetron, tropisetron, MDL 72222), this did not occur. Nevertheless, their experiments in which the neuronal 5-HT transporter was blocked by paroxetine left the possibility open that the facilitatory 5-HT receptors can be activated by endogenous 5-HT when, under certain conditions, its concentration in the synaptic cleft is increased: paroxetine mimicked the effect of 2-methyl-5-HT in that it enhanced the electrically evoked 5-HT release in guinea pig hypothalamic slices, an inhibition which was sensitive to blockade by the 5-HT_1 receptor antagonist tropisetron.

In the in vivo study of MARTIN et al. (1992) on the rat ventral hippocampus, the 5-HT_1 receptor antagonist MDL 72222 did not affect 5-HT release, thus arguing against the possibility that the 5-HT receptors involved are activated by endogenous 5-HT under the conditions applied.

D. Classification

I. Inhibitory Presynaptic 5-HT Autoreceptors

1. Classification in Terms of 5-HT_{1B} and "5-HT_{1D}" Receptors

Until 1990, classification and nomenclature of 5-HT receptors had mainly been based on their operational/transductional behaviour and their pharmacological properties (BRADLEY et al. 1986; LEFF and MARTIN 1988; PEROUTKA 1988; FRAZER et al. 1990). At that time, little information was available on 5-HT receptor structure, since only few genes coding for these receptors had been sequenced; however, the results available from molecular biological studies roughly supported the classification scheme derived from operational data.

Due to the lack of selective ligands at certain 5-HT receptors, these 5-HT receptors, among them the presynaptic 5-HT autoreceptor (see Sect. C.1), could only be classified by application of a large number of 5-HT receptor agonists and antagonists (e.g. ENGEL et al. 1986). In such attempts to classify the presynaptic 5-HT autoreceptors, the potencies of agonists in inhibiting 5-HT release from rat hypothalamic synaptosomes and in rat and pig cerebral cortical slices and of antagonists in antagonizing this inhibition were compared with their potencies in inhibiting binding of appropriate [^{3}H]ligands and [^{125}I]ligands to the various 5-HT-binding sites in membranes from the CNS of several species (MARTIN and SANDERS-BUSH 1982; ENGEL et al. 1983, 1986; SCHLICKER et al. 1989). In subsequent studies which were based on part of these results, relatively few ligands which exhibited a certain, yet limited degree of selectivity for 5-HT receptor subtypes have been applied for classification of the presynaptic 5-HT autoreceptors (e.g. BONANNO et al. 1986; MAURA et al. 1986; for review, see STARKE et al. 1989). Surprisingly, it was found that the presynaptic 5-HT autoreceptor belongs to another subtype than the firing-regulating somadendritic 5-HT autoreceptor: whereas the latter had been characterized as being of the 5-HT_{1A} subtype (SPROUSE and AGHAJANIAN

1986, 1987), the presynaptic 5-HT autoreceptor was classified as 5-HT_{1B} in various regions of the rat CNS and as "5-HT_{1D}" (former nomenclature, labelled by quotation marks) in the pig cerebral cortex (for review and references, see STARKE et al. 1989; nomenclature of "5-HT_{1D}" receptors recently revised, see HARTIG et al. 1996 and below, Sect. D.I.3); furthermore, there were hints that in the guinea pig and rabbit brain the presynaptic 5-HT autoreceptors also possess "5-HT_{1D}" properties.

Although several pharmacological similarities were recognized between rat 5-HT_{1B}-binding sites and/or receptors on the one hand and guinea pig, pig, bovine and human "5-HT_{1D}" sites/receptors on the other, there were some fundamental differences between these subtypes. The most striking one was observed with certain β-adrenoceptor antagonists. Thus, propranolol and cyanopindolol also exhibited rather high affinity for rat 5-HT_{1B}-binding sites ($pK_i > 7$; see footnote to Table 3) and stereoselectively acted as partial agonists exhibiting antagonistic property at rat presynaptic 5-HT autoreceptors [the (−)-enantiomers being more potent]; however, β-adrenoceptor antagonists had a clearly lower affinity for "5-HT_{1D}"-binding sites in the brain of the guinea pig, pig, cow and man (see footnote to Table 3; HEURING and PEROUTKA 1987; WAEBER et al. 1988; 1989). Furthermore, propranolol did not antagonize the inhibitory effect of the 5-HT receptor agonist 5-methoxytryptamine on 5-HT release in the pig cerebral cortex (for review, see STARKE et al. 1989; for details of the investigation in pig cerebral cortex, see SCHLICKER et al. 1989). Another difference between the rat 5-HT_{1B}-binding sites and the "5-HT_{1D}" sites of the other species mentioned was that the 5-HT_{1A} receptor agonist 8-OH-DPAT [8-hydroxy-2(di-*n*-propylamino)tetralin] had very low affinity for the former, but moderate affinity ($pK_i \geq 6$) for "5-HT_{1D}" sites (see footnote to Table 3). Accordingly, 8-OH-DPAT did not inhibit 5-HT release in the rat CNS (see, however, LIMBERGER et al. 1991 and Sect. D.I.4), but behaved as a low-potency agonist at the inhibitory presynaptic 5-HT autoreceptors of the pig cerebral cortex (see STARKE et al. 1989). Other ligands exhibiting a preference in favour of "5-HT_{1D}" receptors are the two rauwolfia alkaloids rauwolscine and yohimbine as well as dipropyl-5-carboxamidotryptamine (see footnotes to Table 3). In recent years, the inhibitory presynaptic 5-HT autoreceptors in various regions of the brain of various species other than rat and pig, including man, have been classified in terms of 5-HT_{1B} and "5-HT_{1D}" (see Tables 1, 3). The similarities in distribution and function between the 5-HT_{1B}- and "5-HT_{1D}"-binding sites/receptors in the various species led HOYER and MIDDLEMISS (1989) to propose that these receptors are species homologues.

2. Subclassification of "5-HT_{1D}" Receptors

Molecular biological studies which revealed that the "5-HT_{1D}" receptors are not homogeneous made it necessary to extend the nomenclature of these receptors (for review, see HARTIG et al. 1992). Two different genes which code for receptors with binding properties virtually identical to those previously

Table 3. Pharmacological properties of the inhibitory presynaptic 5-HT autoreceptors in the brain of various species as a basis for the classification of these receptors in terms of 5-HT_{1B} and "5-HT_{1D}" (last but one column). This "classical" nomenclature was primarily based on the pharmacological differences between 5-HT_{1B}-binding sites in rat brain membranes and "5-HT_{1D}"-binding sites in bovine, guinea pig, porcine and human cerebral membranes (for references, see Sect. D.I.1). These differences were most pronounced with the drugs considered in this table (for references, see footnotes). The classification scheme of the 5-HT_{1D}/"5-HT_{1D}" receptors has recently been revised on

Species	CNS region	References	Potency of 5-OH-DPAT[a]	Potency of yohimbine (Y) or rauwolscine (R)[b]	Potency of dipropyl-5-CT[c]
Rat	Cerebral cortex	MIDDLEMISS and HUTSON (1990)[d]			
	Spinal cord	MATSUMOTO et al. (1992)	$pD_2 < 6$		
		YANG et al. (1994)	(0.1 μM inactive)		
Mouse	Cerebral cortex	SCHIPPER (1990)	("8-OH-DPAT inactive")[e]		
Guinea pig	Cerebral cortex	ORMANDY (1993)			pD_2 6.8
		BUHLEN et al. (1996a) Synaptosomes Slices			
		LIMBERGER et al. (1991)	pD_2 7.06 (partial agonism)	Y: pK_B 6.66 (partial agonism)	
Rabbit	Cerebral cortex	LIMBERGER et al. (1991)	pD_2 6.82 (partial agonism)	Y: pD_2 7.01 (partial agonism)	
	Caudate nucleus	FEUERSTEIN et al. (1992)	(agonism at 0.1 and 0.3 μM)	R. pD_2 about 6.5 (partial agonism)	
Rhesus monkey	Cerebral cortex	SCHIPPER (1990)	pD_2 7.1		
Man	Cerebral cortex	GALZIN et al (1992)	(tendency towards agonism at 0.1 μM)	(agonism by Y 1 μM)	
		MAURA et al. (1993)	(agonism at 0.3 and 1 μM)		

[a] pK_i values of 8-OH-DPAT [8-hydroxy-2(di-n-propylamino)tetralin] in binding studies: 5-HT_{1B} 4.22–5.75; "5-HT_{1D}" 5.90–7.55 (compiled by ZIFA and FILLION 1992).
[b] pK_i values of yohimbine and rauwolscine in binding studies: 5-HT_{1B} 5.46 and 5.25, respectively; "5-HT_{1D}" 7.12 and 7.65, respectively (SCHOEFFTER and HOYER 1990).
[c] pK_i values of dipropyl-5-CT in binding studies: 5-HT_{1B} 4.88; "5-HT_{1D}" 6.92 (SCHOEFFTER and HOYER 1990).
[d] pK_i values of cyanopindolol (CYP) and (–)-propranolol (PRP) in binding studies: 5-HT_{1B} 8.28 and 7.33, respectively; "5-HT_{1D}" 6.85 and 5.49, respectively (SCHOEFFTER and HOYER 1990).
[e] The potencies (pD_2) of agonists [in the study by MIDDLEMISS and HUTSON (1990), also of antagonists, pA_2] at the presynaptic autoreceptors were compared by linear regression analysis with their affinities (pK_i) at 5-HT_{1B}- and "5-HT_{1D}"-binding sites (n, number of drugs). Either the regression analyses were

the basis of the primacy of the human genome (last column: new nomenclature according to Hartig et al. 1996), taking into account that all presynaptic 5-HT autoreceptors listed in this table are probably encoded by orthologous genes (see Sects. D.1.3, 4). This table is restricted to more recent investigations not yet considered in the review by Starke et al. (1989). Slices or synaptosomes preincubated with [^{3}H] 5-HT were superfused and [^{3}H] 5-HT release was evoked by electrical field stimulation or high K^+. In each study the inhibitory effect of 5-HT or 5-carboxamidotryptamine 5-CT on the evoked [^{3}H] 5-HT release was counteracted by methiothepin or metergoline

Potency of cyanopindolol (CYP) or propranolol (PRP)[a]	Correlation coefficients (r), comparison of potencies of ligands with affinities for binding sites[b]		Classification	
			Primacy of pharmacology[c]	Primacy of genome[d]
	5-HT_{1B}	"5-HT_{1D}"		
CYP: pA_2 8.4	0.87 (n = 12)	0.32 (n = 11)	5-HT_{1B}[e]	r5-HT_{1B}
	0.79 (n = 8)	0.71 (n = 7)	5-HT_{1B}	r5-HT_{1B}
CYP: pA_2 8.3			5-HT_{1B}	m5-HT_{1B}
	0.87 (n = 5)	0.88 (n = 5)	"5-HT_{1D}"	gp5-HT_{1B}
	0.78 (n = 6) 0.55 (n = 6)	0.86 (n = 6) 0.82 (n = 6)		
PRP pA_2 < 6, pD_2 < 5[f]	0.76 (n = 4)	0.95 (n = 4)		
PRP: pA_2 < 6, pD_2 < 6[g]	0.84 (n = 5)	0.94 (n = 5)	"5-HT_{1D}"	rb5-HT_{1B}
(agonism by CYP 1 and 10 μM)			"5-HT_{1D}"	rb5-HT_{1B}
CYP, pA_2 6.5			"5-HT_{1D}"	mk5-HT_{1B}
(antagonism by PRP 1 μM)			"5-HT_{1D}"	h5-HT_{1B}

carried out by the authors themselves or by us, using the potencies given by the authors quoted in this table and the pK_i values reported by Schoeffter and Hoyer (1990, 1991), Hoyer and Schoeffter (1991) and Beer et al. (1993).

[b] "Classical" nomenclature in terms of 5-HT_{1B} and "5-HT_{1D}" (see Sect. D.1.1).

[c] New nomenclature (see Sects. D.1.3, 4).

[d] pD_2, pA_2 and pK_i values were assessed from Fig. 4 of that paper.

[e] The same conclusion was previously drawn by Engel et al. (1986). The study by Limberger et al. (1991), however, provides evidence that, in addition, an r5-HT_{1E} receptor is involved (see Sect. D.1.4).

[f] No exact concentration range given in that paper.

[g] pD_2 for partial agonism.

determined for "5-HT_{1D}"-binding sites in human cerebral membranes, but different from rat 5-HT_{1B} sites, were isolated from the human brain. These receptors were called "5-$HT_{1D\alpha}$" and "5-$HT_{1D\beta}$". Whereas they exhibited almost identical binding characteristics for 19 different 5-HT receptor agonists and antagonists (see Hartig et al. 1992), they can be distinguished by ketanserin and, less well, by ritanserin, which have 75- and 24-fold higher affinity, respectively, for cloned human "5-$HT_{1D\alpha}$" than "5-$HT_{1D\beta}$" receptors (Zgombick et al. 1995).

Accordingly, ketanserin has been used to determine the "5-HT_{1D}" receptor subtype to which the presynaptic 5-HT autoreceptors belong in the human (Fink et al. 1995 and Fig. 2) and guinea pig cerebral cortex (Bühlen et al. 1996a): they were subclassified as "5-$HT_{1D\beta}$" and "5-$HT_{1D\beta}$-like", respectively (for more details see below, Sect. D.I.4).

The gene which encodes the human "5-$HT_{1D\beta}$" receptor is highly homologous to a gene which was isolated from the rat brain and which was found to code for a receptor with the pharmacological characteristics of the 5-HT_{1B}-binding sites in rat cerebral membranes (Hartig et al. 1992). These findings strongly supported the suggestion that, in spite of the pharmacological differences mentioned above, the presynaptic 5-HT_{1B} autoreceptor in the rat and the presynaptic "5-HT_{1D}" autoreceptor in other species such as the guinea pig and man are species homologues (see above; Hoyer and Middlemiss 1989): only few differences in the amino acid sequence are responsible for the differences in pharmacological profile (Metcalf et al. 1992; Oksenberg et al. 1992; Parker et al. 1993).

In addition to the gene encoding the rat 5-HT_{1B} receptor, a rat homologue of the gene encoding the human 5-$HT_{1D\alpha}$ receptor was identified; this rat gene coded for a receptor with the binding properties of a "5-HT_{1D}" receptor (Bach et al. 1993).

3. Revised Nomenclature of 5-$HT_{1B/1D}$ Receptors on the Basis of the Primacy of the Human Genome

As a result of the history of the discovery of genes, binding sites and receptors of the "5-$HT_{1B/1D}$" subtype, a complex and confusing nomenclature of this receptor subfamily has developed (Göthert 1992; Zifa and Fillion 1992; Boess and Martin 1994; Hoyer et al. 1994; Martin and Humphrey 1994). Therefore, the nomenclature has recently been revised and simplified on the basis of the primacy of the human genome (Hartig et al. 1996) taking the following general rules, accepted by the International Union of Pharmacology Committee for Receptor Nomenclature, into account: a name should first be established for the amino acid sequence of a receptor subtype deduced from a certain human gene. The same name should be used for all species homologues of this receptor subtype or, in other words, for all receptors which are the products of orthologous genes, irrespective of whether or not the pharmacological properties of these receptors are identical. The species should be

specified by brief letter prefixes (e.g. "h" for human, "mk" for monkey, "r" for rat, "m" for mouse, "gp" for guinea pig, "rb" for rabbit, "p" for pig; VANHOUTTE et al. 1996). For instance, taking these principles into account, the human "5-$HT_{1D\beta}$" and "5-$HT_{1D\alpha}$" receptors have been renamed h5-HT_{1B} and h5-HT_{1D}, respectively, and analogously the rat 5-HT_{1B} and "5-HT_{1D}" receptors are termed r5-HT_{1B} and r5-HT_{1D}, respectively. Thus, it becomes obvious at first sight that the h5-HT_{1B} and r5-HT_{1B} receptors belong to the same subtype in spite of their pharmacological differences.

It is also clear from the previous sections that the new nomenclature has more far-reaching consequences for the terminology of the presynaptic 5-HT autoreceptors than for any other functional 5-HT receptors described so far. Therefore, the development of the nomenclature had to be described here in some detail in order to enable access from this review to the underlying original work on these receptors.

4. Subclassification and Nomenclature in Terms of the Revised Nomenclature

As outlined above and in Table 3, the presynaptic 5-HT autoreceptors have originally been classified as 5-HT_{1B} in rats and mice and as "5-HT_{1D}" (according to the nomenclature introduced before 1989) in the other species investigated up to now. It has also been mentioned that, in view of the heterogeneity of the latter receptors, attempts have recently been made in guinea pig and human brain slices and synaptosomes to subclassify the presynaptic 5-HT autoreceptors (Sect. D.I.2). Some details of these studies, which appeared before the new nomenclature was published, are worthwhile to be reported here. In order to avoid confusion, the primacy of the new nomenclature is emphasized by mentioning the new name either alone or first, the old nomenclature being added in some cases in quotation marks.

It has already been stated above (Sect. D.I.2) that subclassification of human presynaptic 5-HT autoreceptors was complicated by the lack of compounds which discriminate between h5-HT_{1B} ("5-$HT_{1D\beta}$") and h5-HT_{1D} ("5-$HT_{1D\alpha}$") receptors and that among the drugs available ketanserin was most suitable in this respect. In a study in human cortical synaptosomes (FINK et al. 1995; GÖTHERT et al. 1996), ketanserin at a concentration 4.4 times higher than its K_i at cloned h5-HT_{1D} receptors but more than 16 times lower than its K_i at cloned h5-HT_{1B} receptors failed to antagonize the inhibitory effect of 5-CT on the K^+-evoked [^{3}H]5-HT release (Fig. 2). In contrast, the non-selective antagonist at h5-$HT_{1B/1D}$ receptors, methiothepin, at a concentration 4.4 times higher than its K_i at cloned h5-HT_{1B} receptors abolished the 5-HT-induced inhibition (Fig. 2). This pattern of effects of the two antagonists made it possible to conclude that the presynaptic inhibitory 5-HT autoreceptor of the human cerebral cortex belongs to the h5-HT_{1B} ("5-$HT_{1D\beta}$"; Sect. D.I.2) subtype.

Very recently two new drugs SB 216641 (*N*-[3-(2-dimethylamino)ethoxy-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1′-

biphenyl)-4-carboxamide) and BRL 15572 (1-phenyl-3-[4-(3-chlorophenyl) piperazin-1-yl]phenylpropan-2-ol), which discriminate between h5-HT_{1B} and h5-HT_{1D} receptors, have become available: SB 216641 exhibited 30-fold higher affinity for cloned h5-HT_{1B} receptors than for cloned h5-HT_{1D} receptors, whereas BRL 15572 had over 100-fold greater affinity for cloned h5-HT_{1D} than for cloned h5-HT_{1B} receptors (Price et al. 1996). In human cerebral cortical synaptosomes, the inhibitory effect of 5-HT on the K^+-evoked 5-HT release was antagonized by SB 216641, but not affected by BRL 15572 (both drugs applied at concentrations 15 times higher than their K_i at cloned h5-HT_{1B} and h5-HT_{1D} receptors, respectively: Schlicker et al. 1997). These data strongly support the conclusion that the presynaptic 5-HT autoreceptor in the human cerebral cortex is of the 5-HT_{1B} subtype. In agreement with this, SB 216641, but not BRL 15572, increased the electrically evoked 5-HT release from human cerebral cortical slices.

Basically the same approach as in human brain preparations was used to subclassify the presynaptic 5-HT autoreceptor in the guinea pig cerebral cortex, although the gp5-HT_{1B} and gp5-HT_{1D} receptors have not yet been cloned. However, the pharmacological properties of the presynaptic 5-HT autoreceptors in the guinea pig cerebral cortex (Limberger et al. 1991; Bühlen et al. 1996a; Roberts et al. 1996) are similar to those in the human cerebral cortex (Schlicker et al. 1985; Galzin et al. 1992; Matuka et al. 1993) and the same holds true for the "5-HT_{1D}"-binding sites in cerebral membranes from both species (Waeber et al. 1988, 1989; Beer et al. 1992; Bruinvels et al. 1992). Therefore, it is generally accepted that the guinea pig brain is suitable as an experimental model for the development of autoreceptor ligands which may become useful as therapeutic compounds in human diseases (see below). To provide evidence that in the guinea pig cerebral cortex the presynaptic 5-HT autoreceptor is similar to the h5-HT_{1B} receptor ("5-$HT_{1D\beta}$"-like), a study was carried out in guinea pig cortical synaptosomes in which ketanserin and methiothepin were applied at appropriate concentrations (Bühlen et al. 1996a). In fact it was found that the inhibitory effect of 5-CT on 5-HT release was not affected by ketanserin at a concentration 4 times higher than its K_i at cloned h5-HT_{1D} ("5-$HT_{1D\alpha}$") receptors, but that methiothepin, at a concentration 4 times higher than its K_i value at h5-HT_{1B} ("5-$HT_{1D\beta}$") receptors, caused a rightward shift of the concentration-response curve of 5-CT.

Further support for the suggestion that the presynaptic 5-HT autoreceptors in the guinea pig brain predominantly are h5-HT_{1B}-like came from binding studies in cortical synaptosomes and membranes from this species. In the synaptosomal preparation, the fraction of presynaptic membranes related to the total quantity may be assumed to be higher than in conventionally prepared membranes. Whereas the pharmacological properties of the [^{3}H]5-HT binding sites in membranes correlated with those of both h5-HT_{1B} and h5 HT_{1D} receptors, the pharmacological characteristics of the [^{3}H]5-HT-binding sites in synaptosomes correlated with those of h5-HT_{1B} ("5-$HT_{1D\beta}$") only, but not with those of h5-HT_{1D} receptors ("5-$HT_{1D\alpha}$"; Bühlen et al.

1996a). In both preparations there was no correlation with the properties of the other 5-HT receptors within the seven 5-HT receptor families which have so far been identified in the guinea pig or other species.

On the basis of the observation that the presynaptic 5-HT autoreceptor belongs to the 5-HT_{1B} subclass of the new classification scheme not only in rat and mouse but also in guinea pig and man, it may be suggested that this probably also holds true for the other species in which they have previously been classified as "5-HT_{1D}", i.e. for pig (Schlicker et al. 1989), rhesus monkey and rabbit (Table 3; the rb5-HT_{1B} receptor has recently been cloned; Baxd et al. 1996).

However, this general rule does not implicate that all presynaptic 5-HT receptors are of the 5-HT_{1B} subtype. Thus, the presynaptic inhibitory 5-HT heteroreceptors on the sympathetic nerve fibres innervating the human atrial appendages have been classified by means of ketanserin as h5-HT_{1D} ("5-$HT_{1D\alpha}$": Göthert et al. 1996; Molderings et al. 1996). Another example refers to the presynaptic 5-HT autoreceptors in the rat brain. According to experiments by Limberger et al. (1991) in cerebral cortical slices, not all of these receptors belong to the r5-HT_{1B} subtype, but a part of them could be subclassified as r5-HT_{1D}; whereas the effects of most drugs conformed to the r5-HT_{1B} character of the presynaptic 5-HT autoreceptor (e.g. the antagonism by the preferential 5-HT_{1B} receptor antagonist isamoltane of the inhibitory effect of RU 24969 [5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole] on [^{3}H]5-HT release), the inhibitory effect of 8-OH-DPAT in the high nanomolar range did not. The potency of this compound was virtually identical to that in guinea pig or rabbit cortical slices examined in the same study. The effect of 8-OH-DPAT was counteracted by methiothepin but not by isamoltane. These findings with 8-OH-DPAT are compatible with the involvement of r5-HT_{1D} receptors.

In the guinea pig hippocampus, two different presynaptic 5-HT autoreceptors appear to occur as well (Wilkinson and Middlemiss 1992): in slices of this brain region methiothepin antagonized the effect of 5-HT at higher potency (apparent pA_2 value 7.6) than the effects of 5-CT and sumatriptan (pA_2 6.7–7.0). It is by no means clear whether these findings can be accounted for by the occurrence of both the gp5-HT_{1B} and the gp5-HT_{1D} receptor or of another 5-HT receptor in addition to the gp5-HT_{1B} receptor. The operation of multiple presynaptic 5-HT autoreceptors has recently been confirmed in the guinea pig cerebral cortex by analysis of the concentration response curves for 5-HT and 5-CT and of the antagonistic property of GR 127935 [2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide] (Roberts et al. 1996).

Heterogeneity of 5-HT receptors modulating 5-HT release has recently been proved in cortical and hippocampal slices from the m5-HT_{1B} receptor "knock-out" mice (Pineyro et al. 1995b) generated by Saudou et al. (1994). It was found that the r5-HT_{1B} receptor agonist CP-93129 [3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one] and the 5-$HT_{1B/1D}$ receptor

agonist sumatriptan did not inhibit the electrically evoked 5-HT release, whereas the less selective compound 5-CT was still capable of decreasing 5-HT release. In fact, 5-CT has relatively high affinity not only for 5-HT_{1B} and 5-HT_{1D} receptors but also for 5-HT_5 and 5-HT_7 receptors (Hoyer et al. 1994). Therefore, the results with 5-CT suggest that, at least in the absence of m5-HT_{1B} receptors, autoinhibition of 5-HT release may be mediated by m5-HT_5 and/or m5-HT_7 receptors (Pineyro et al. 1995b). It is conceivable that the inhibitory effect mediated via other than m5-HT_{1B} receptors may play a more important role in m5-HT_{1B} receptor "knock-out" animals than under normal conditions: these receptors may be expressed at higher density as a compensatory mechanism. The location of such non-5-HT_{1B} receptors is not clear. They may either represent true presynaptic 5-HT autoreceptors or they may be located on non-serotoninergic neurons within a short regulatory neuronal loop (see Sect. A).

The new nomenclature of 5-$HT_{1B/1D}$ receptors offers a further advantage, namely that allelic polymorphism as a basis of structural variants of receptor subtypes can easily be dealt with in this classification scheme (Hartig et al. 1996). Recently, a coding mutation in nucleotide position 371 (T→G substitution) of the h5-HT_{1B} receptor gene was found in single-strand conformation analysis of the h5-HT_{1B} receptor gene from healthy persons for DNA sequence variation. This mutation leads to an amino acid exchange (Phe→Cys) in position 124 of the receptor protein in the third transmembrane domain close to the junction with the extracellular loop (Nöthen et al. 1995). In membranes from cultured COS-7 cells transfected with the cDNA of the wild-type or mutant h5-HT_{1B} receptor, binding of [^{3}H]5-CT was determined (Bühlen et al. 1996b,c). In saturation experiments the affinity of [^{3}H]5-CT for the mutant h5-HT_{1B} receptor was higher, whereas B_{max} of this receptor was lower compared to the wild-type receptor. Competition experiments with eight full or partial 5-HT_{1B} receptor agonists including 5-HT, dihydroergotamine, sumatriptan and methysergide revealed that their inhibitory potencies were 0.3–0.5 log units higher than for the wild-type receptor. Corresponding changes in the pharmacological properties of the h5-HT_{1B} receptor may be assumed to occur in individuals in whom the mutant h5-HT_{1B} gene is expressed as presynaptic 5-HT autoreceptor: the increased affinity of the endogenous agonist 5-HT and of other 5-HT_{1B} receptor ligands may lead to a decrease in 5-HT release (by increased autoreceptor function) and to pharmacogenetic differences in the action of 5-HT_{1B} receptor ligands.

II. 5-HT Receptors Mediating Facilitation of 5-HT Release

The facilitatory 5-HT receptors identified in the guinea pig and rat brain in vitro (Galzin and Langer 1991; Blier and Bouchard 1993) and in vivo (Martin et al. 1992) belong to the 5-HT_3 receptor class. This can be deduced mainly from the findings with the 5-HT_3 receptor agonists and antagonists mentioned above (Sect. C.II.1). When taking the species differences in the

pharmacological properties of 5-HT_3 receptors into account (for review see KILPATRICK et al. 1990; HOYER et al. 1994; Chap. 18, this volume), the facilitatory 5-HT receptor in the guinea pig brain (cerebral cortex, hippocampus and hypothalamus) also fulfilled the criteria characteristic for the 5-HT_3 receptor of this species (BLIER and BOUCHARD 1993): the agonists phenylbiguanide and m-chlorophenylbiguanide, which have been shown to be effective 5-HT_3 receptor agonists in rat but not guinea pig tissues (KILPATRICK et al. 1991; ROBERTSON and BEVAN 1991; BUTLER et al. 1990), failed to mimic the facilitatory effect of 2-methyl-5-HT; in agreement with the general properties of 5-HT_3 receptors in guinea pig tissues (for references, see above), the potencies of the 5-HT_3 receptor antagonists were generally low, (+)-tubocurarine even being ineffective.

In agreement with the property of 5-HT_3 receptors to desensitize in response to prolonged stimulation (PETERS and LAMBERT 1989; YAKEL 1992; Chap. 18, this volume), it was observed in rat hypothalamic and guinea pig cerebral cortical and hypothalamic slices that 2-methyl-5-HT facilitated 5-HT release only if the time of exposure was 5 min but not if it was extended to 20 min (GALZIN and LANGER 1991; BLIER and BOUCHARD 1993). However, in this context it should be noted that in electrophysiological experiments, 5-HT_3 receptors proved to desensitize much faster (YAKEL and JACKSON 1988; YAKEL 1992).

E. Site and Mechanism of Action

I. Inhibitory Presynaptic 5-HT Autoreceptors

From early work carried out before 1989 only few, rather general conclusions could be drawn concerning the ionic or biochemical events induced in the cell membrane by activation of inhibitory presynaptic 5-HT autoreceptors (for review and references, see STARKE et al. 1989). Propagation of action potentials seems not to be affected, but stimulus-release coupling, in particular transmembrane Ca^{2+} influx into the serotoninergic axon terminals, appears to be inhibited. The results of experiments designed to investigate the involvement of G proteins in autoreceptor-mediated effects were contradictory. Whereas PASSARELLI et al. (1988) found the effect of two 5-HT receptor agonists on 5-HT release in rat hippocampal slices to be pertussis toxin-sensitive, the data obtained in interaction experiments of 5-HT receptor ligands with pharmacological tools known to influence the adenylyl cyclase (SCHLICKER et al. 1987, rat brain cortex slices) or protein kinase C second messenger system (FEUERSTEIN et al. 1987, rabbit hippocampal slices; WANG and FRIEDMAN 1987, rat brain cortex slices) argued against inhibitory coupling of the autoreceptors to these biochemical systems via G proteins.

Although it is now clear that the 5-HT_{1B} receptor and, accordingly, the presynaptic 5-HT autoreceptor belongs to the superfamily of G-protein-

coupled receptors (Hoyer et al. 1994), the experimental evidence for the involvement of G proteins and adenylyl cyclase or protein kinase C remained equivocal. Blier (1991) studied the effect of two inhibitors of G_{α} proteins, pertussis toxin and *N*-ethylmaleimide (NEM), and of a stimulator of G_s proteins, cholera toxin, in rat hippocampal slices. Pertussis and cholera toxin, with which the rats were pretreated, and which by themselves did not change 5-HT release, failed to modify the effect of 5-methoxytryptamine, 5-CT and/or methiothepin on 5-HT release. NEM, which by itself increased 5-HT release, also did not change the effect of 5-methoxytryptamine. In guinea pig brain cortex slices, forskolin (an activator of adenylyl cyclase), rolipram (an inhibitor of cAMP phosphodiesterase) and 8-Br-cAMP (a lipid-soluble analogue of cAMP) did not affect 5-HT release when given alone, but attenuated the modulatory effect of 5-CT and/or sumatriptan on 5-HT release (Ormandy 1993). 1,9-Dideoxyforskolin, which fails to activate adenylyl cyclase (but, like forskolin, blocks K^+ channels), did not attenuate the effect of 5-CT. In rat hypothalamic slices, forskolin, 8-Br-cAMP and an inhibitor of phospodiesterase (IBMX) both increased 5-HT release, when given alone, and did not attenuate the inhibitory effects of 5-methoxytryptamine and RU 24969 on 5-HT release (Ramdine et al. 1989). In contrast, inhibition of protein kinase C by phorbol-11,12-dibutyrate (which by itself also increased 5-HT release) attenuated the modulatory effects of 5-methoxytryptamine and methiothepine on 5-HT release.

Taken together, the inconsistencies observed in these experiments on cerebral slices are probably due to the fact that the methods applied so far have been too indirect. More direct approaches in future studies will probably provide more unequivocal results.

II. 5-HT Receptors Mediating Facilitation of 5-HT Release

The ionic and biochemical effects in the cell membrane induced by activation of the 5-HT receptor mediating facilitation of 5-HT release are even less well known. This is already evident when considering that the exact location of the receptor is still unclear. As mentioned above (Sects. A, C.II.1), the 5-HT receptor is probably located on a non-serotoninergic interneuron. Hence, in a cerebral slice preparation, an event induced by activation of such a receptor may occur in any of the nerves involved in the facilitatory neuronal circuit.

On the basis of the investigation by Blier and Bouchard (1993) on guinea pig hypothalamic slices, it appears that an increase in transmembrane influx of Ca^{2+} ions is a prerequisite for the 5-HT_3 receptor-mediated facilitation: the facilitatory effect of 2-methyl-5-HT on 5-HT release only occurred when 5-HT release was stimulated by Ca^{2+}-dependent methods of stimulation such as electrical impulses or high K^+, whereas the Ca^{2+}-independent fenfluramine-induced 5-HT release was not altered.

F. Plasticity of Inhibitory Presynaptic 5-HT Autoreceptors

Very recently it has been shown that the functional activity of the presynaptic 5-HT autoreceptor can be modulated by an endogenous tetrapeptide, Leu-Ser-Ala-Leu (LSAL), which was called 5-HT-moduline (Massot et al. 1996; Chap. 11, this volume). This peptide is released from synaptosomes and binds to specific recognition sites in mammalian brain and in transfected cells expressing 5-HT_{1B} receptors. In rat hippocampal synaptosomes, interaction of 5-HT-moduline with presynaptic 5-HT_{1B} autoreceptors was shown to decrease, and at appropriate concentration even abolish, the inhibitory effect of a 5-HT_{1B} receptor agonist on evoked 5 HT release.

In numerous investigations (see below), the possibility has been examined that the sensitivity of inhibitory 5-HT autoreceptors may change with the light-dark cycle, aging and chronic treatment with drugs, in particular antidepressant drugs, or electroconvulsive shocks (ECS).

I. Lack of Circadian Variations

Many of the steps involved in the synthesis and release of 5-HT are subject to circadian variations (Martin 1991). For example, the release of 5-HT measured by in vivo microdialysis in the hippocampus of freely moving rats undergoes changes during the light-dark cycle (Kalen et al. 1989), a phenomenon which might be related to variations of the sensitivity of the inhibitory 5-HT autoreceptor. This possibility was examined in experiments of Singh and Reuterkn (1994a,b), who determined 5-HT release and its autoreceptor-mediated modulation in slices from the rat cerebral cortex and hippocampus and from the guinea pig cerebral cortex at four equally spaced time points in the 12-h light/12-h dark cycle (end-dark, mid-light, end-light, mid-dark). The potencies of exogenous 5-HT in inhibiting 5-HT release and of methiothepin in antagonizing the effect of endogenous or exogenous 5-HT did not differ between the four time points. In another study, Blier et al. (1989) compared 5-HT release and its autoreceptor-mediated modulation in hypothalamic slices prepared from two groups of rats subjected to a 12-h light/12-h dark cycle. In the first group, in which the lights were turned on at 7h and off at 19h, 5-HT release was higher than in the second group, in which the lights were turned on at 19h and off at 7h. However, both groups did not differ with respect to the inhibitory effect of 5-methoxytryptamine and RU 24969 and the facilitatory effect of methiothepin on 5-HT release. Thus, circadian variations of the sensitivity of inhibitory 5-HT autoreceptors do not appear to occur.

II. Age-Dependent Variations

The density of various types of 5-HT-binding sites in the brain has been reported to undergo alterations during aging (for review, see Amenta et al.

1991). Therefore, the possibility that the inhibitory 5-HT autoreceptor may also undergo age-dependent alterations was examined in two in vitro studies. In cerebral cortex slices from 3-month-old rats, 5-HT release was higher than in slices from 2.5-year-old animals, but the potency of exogenous 5-HT in inhibiting 5-HT release and the potency of methiothepin in antagonizing the inhibitory effect of endogenous and exogenously 5-HT was identical in both groups (Schlicker et al. 1989). In spinal cord slices from 3-month-old rats, 5-HT and mCPP [1-(3-chlorophenyl)piperazine] inhibited 5-HT release, whereas in slices from 18- to 20-month-old animals, both compounds facilitated 5-HT release (release in the absence of the 5-HT receptor agonists not mentioned; Murphy and Zemlan 1989).

III. Changes by Drugs and Electroconvulsive Shocks

Long-term treatment of patients suffering from affective disorders with tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), reversible or irreversible monoamine oxidase (MAO) inhibitors and electroconvulsive shocks may cause alterations of the sensitivity of the inhibitory 5-HT autoreceptor. Such changes might be due to the increased concentration of 5-HT in the synaptic cleft as a consequence of reduced inactivation or increased release of 5-HT. This possibility was studied in animals treated as mentioned, usually for 2–3 weeks (see Table 4 for such in vitro studies; as an exception, this table also contains data obtained before 1989). Furthermore, the possibility was investigated in animals that blockade of the presynaptic 5-HT autoreceptors with methiothepin or treatment with nimodipine, a Ca^{2+} channel blocker potentially leading to a decreased 5-HT release, might cause an increased sensitivity of the autoreceptors (Table 4).

The sensitivity of the autoreceptor was examined in functional experiments in vitro, i.e. by investigating the effects of agonists and/or antagonists on 5-HT release. Therefore, an important prerequisite for such investigations is that, at the time of determination of autoreceptor function, no residual compound used for long-term treatment is left in the tissue. Accordingly, in all studies entered into Table 4, administration of the drug was stopped 17–72 h before sacrifice and in some of them the absence of residual compound was proven by demonstrating that 5-HT uptake or MAO was no longer inhibited. In order to make sure that a change in sensitivity after long-term treatment with a drug is different from its acute effect, the sensitivity of autoreceptors should also be examined after 1- or 2-day administration. This has been examined in some, but not all, studies listed in Table 4. Ideally, the recovery from long-term treatment-induced change in sensitivity after withdrawal of the drug should also be demonstrated [as was done by Maura and Raiteri (1984) only; Table 4].

In analogy to the time schedule applied in the experiments with long-term treatment with drugs, the sensitivity of the release-modulating receptors was

Table 4. Effect of long-term treatment with various drugs or electroconvulsive shocks on the sensitivity of inhibitory 5-HT autoreceptors. "0", "+" and "–" means that the long-term treatment did not affect, increase or decrease, respectively, the sensitivity of the 5-HT receptors. "Acute treatment" means that the drug was given for 1 or 2 day(s) only

Species, brain region Authors; preparation; stimulus	Treatment schedule	Change in sensitivity of the autoreceptor (in parentheses, drug(s) used to test sensitivity)	Comment
Rat, cerebral cortex			
Schlicker et al. (1991); slices; electrical pulses	Nimodipine 65 mg/kg in food pellets for at least 6 weeks	0 (5-HT)	5-HT release not affected by chronic nimodipine
Maura and Raiteri (1984); synaptosomes; high K^+	CGP 6085A 10 mg/kg i.p. plus clorgyline 1 mg/kg i.p. for 15 days	– (5-HT)	5-HT release not affected by the two chronic treatment schedules
	Methiothepin 10 mg/kg i.p. for 15 days	+ (5-HT)	Acute treatment with CGP 6085A plus clorgyline or methiothepin did not affect autoreceptor sensitivity
			Autoreceptor sensitivity recovered 10 days after withdrawal from chronic CGP 6085A + clorgyline
Rat, hypothalamus			
Hagan and Hughes (1983); slices; electrical pulses	Methiothepine 10 mg/kg i.p. for 21 days	0 (5-HT, methiothepin)	5-HT release and uptake not affected by chronic methiothepin
Oxford and Warwick (1987); slices; high K^+	Nialamide 40 mg/kg i.p. or clomipramine 10 mg/kg i.p. for 13 days each	– (5-MeOT)	5-HT release increased after chronic nialamide but
		0 (5-MeOT)	not affected after chronic clomipramine
			Acute treatment with nialamide did not affect autoreceptor sensitivity
Mobley and Briley (1990); slices; electrical pulses	Citalopram 10 mg/kg in the drinking water or	– (LSD)	5-HT release increased after chronic citalopram (50 > 10 mg/kg) but not affected after chronic milnacipran
	citalopram 50 mg/kg in food pellets or	– (LSD)	
	milnacipran 50 mg/kg in food pellets for 21 days each	0 (LSD)	Effect of acute treatment with citalopram on autoreceptor sensitivity not examined

Table 4. *Continued*

Species, brain region Authors; preparation; stimulus	Treatment schedule	Change in sensitivity of the autoreceptor (in parentheses, drug(s) used to test sensitivity)	Comment
Schoups and de Potter (1988); slices; electrical pulses	Amitriptyline 10mg/kg i.p. for 21 days	– (5-HT, methiothepin)	5-HT release increased by chronic amitriptyline Effect of acute treatment with amitriptyline on autoreceptor sensitivity not examined
Guinea pig, frontal cortex hippocampus and hypothalamus			
Blier and Bouchard (1994); slices; electrical pulses	Paroxetine 10mg/kg[a] for 21 days	0 or –[b]	Residual 5-HT uptake or MAO blockade after chronic paroxetine and befloxatone excluded
	Befloxatone 0.75mg/kg[a] for 21 days	0 (5-MeOT, methiothepin)	5-HT release increased after chronic paroxetine or befloxatone Effect of acute treatment with paroxetine on the sensitivity of the inhibitory autoreceptor has not been studied
Guinea pig, frontal cortex			
el Mansari et al. (1995); slices; electrical pulses	Paroxetine 10mg/kg[a] for 3 or 8 weeks	0 (5-MeOT)	5-HT release increased by chronic paroxetine (3 or 8 weeks) 5-HT transporter desensitized by chronic paroxetine (8 weeks) No residual inhibition of 5-HT uptake after chronic paroxetine (8 weeks)

Guinea pig, orbitofrontal cortex EL MANSARI et al. (1995); slices; electrical pulses	Paroxetine 10mg/kg[a] for 3 weeks, for 8 weeks Fluoxetine 5mg/kg[a] for 3 or 8 weeks	0 (5-MeOT) – (5-MeOT) 0 (5-MeOT)	5-HT release increased by chronic paroxetine (8 weeks) but not by chronic paroxetine (3 weeks) and chronic fluoxetine (3 or 8 weeks) 5-HT transporter not desensitized by chronic paroxetine or fluoxetine (8 weeks each)
Guinea pig, caudate nucleus EL MANSARI et al. (1995); slices; electrical pulses	Paroxetine 10mg/kg[a] or fluoxetine 5mg/kg[a] for 3 or 8 weeks each	0 (5-MeOT)	5-HT release not increased by chronic paroxetine or fluoxetine (3 or 8 weeks each)
Guinea pig, hypothalamus BLIER and BOUCHARD (1992); slices; electrical pulses	Electroconvulsive shock three times per week for 14 days	0 (5-CT, methiothepin)	5-HT release not affected after repeated administration of electroconvulsive shocks Same treatment schedule attenuated the 8-OH-DPAT-induced hypothermia in rats in the same study
Rabbit, hypothalamus SCHOUPS and DE POTTER (1988); slices; electrical pulses	Amitriptyline 20mg/kg i.p. or clomipramine 10mg/kg i.p. or imipramine 20mg/kg i.p. or mianserin 20mg/kg i.p. for 21 days each	0 (5-HT) – (methiothepin)	5-HT release not affected by chronic amitriptyline, clomipramine or imipramine but decreased by chronic mianserin Effect of acute treatment with the four drugs on autoreceptor sensitivity not examined Amitriptyline 10mg/kg i.p. for 21 days reduced the autoreceptor sensitivity in the rat hypothalamus (see above)

[a]Administered via osmotic minipump.
[b]Sensitivity of the inhibitory autoreceptor was not changed in the cerebral cortex (test drug: 5-MeOT) but decreased in the hippocampus (test drugs: 5-MeOT, methiothepin) and hypothalamus (test drugs: 5-MeOT, 5-CT, methiothepin).
CGP 6085A, 4-(5,6-dimethyl-2-benzofuranyl)piperidine; 5-CT, 5-carboxamidotryptamine; 5-MeOT, 5-methoxytryptamine; 8-OH-DPAT, 8-hydroxy-2(di-n-propylamino)tetralin.

examined 48h after the last of a series of six electroconvulsive shocks over a 2-week period (Table 4; Blier and Bouchard 1992).

Table 4 indicates that in some, but not all, studies of long-term treatment with tricyclics, SSRI, MAO inhibitors and electroconvulsive shocks, the expected decrease in sensitivity of inhibitory 5-HT autoreceptors was observed. An increase in sensitivity of the inhibitory 5-HT autoreceptors which was expected after their long-term blockade with methetiothepin was found only by Maura and Raiteri (1984), but not by Hagan and Hughes (1983; see Table 4).

An increase in 5-HT release is another effect which could be predicted for experiments in slices (but not in synaptosomes; see Sect. C.I.2) if long-term treatment of animals with antidepressant drugs led to a decreased function of the inhibitory 5-HT autoreceptors. Since the autoreceptors are normally tonically activated, this should occur because of the impaired negative feedback loop. Indeed such an increase in 5-HT release was found in several of the studies summarized in Table 4 (Oxford and Warwick 1987; Schoups and de Potter 1988; Moret and Briley 1990; Blier and Bouchard 1994; El Mansari and Blier 1996), but not in synaptosomes (Maura and Raiteri 1984).

The effect of long-term treatment with SSRI and/or dexfenfluramine on the sensitivity of inhibitory 5-HT autoreceptors was also examined in vivo. Gardier et al. (1992) injected rats with fluoxetine 30mg/kg i.p. or dexfenfluramine 7.5mg/kg i.p. for 3 days, and 24h after the last injection they determined the effect of methiothepin administered via the dialysis probe on the basal and high K^+-induced 5-HT release in the frontal cortex of the animals anaesthetized with α-chloralose/urethane. After both fluoxetine and dexfenfluramine treatment, the basal and K^+-induced 5-HT release was decreased; the facilitatory effect of methiothepin on the basal 5-HT release was not affected whereas the facilitatory effect on the evoked 5-HT release was attenuated (effect of an acute administration of the drugs not examined). In the study of Bosker et al. (1995), the rats received fluvoxamine 6.7mg/kg daily via an osmotic minipump for 21 days, leading to a fluvoxamine brain level corresponding to its IC_{50} for inhibition of the synaptosomal 5-HT transporter. Three days after removal of the minipump, they measured the effect of RU 24969, administered via the dialysis probe, on basal 5-HT release in the dorsal hippocampus of the freely moving animals. Both 5-HT release and its inhibition by RU 24969 were unaltered. Auerbach and Hjorth (1995) treated rats with citalopram, either with 2 × 5mg/kg s.c. for 2, 7 or 14 days, with 2 × 20mg/kg s.c. for 14 days, or with 10mg/kg via an osmotic minipump for 14 days. Twenty-four hours after the last injection or removal of the minipump, citalopram and later, in addition, RU 24969 or CP-93,129 were administered via the dialysis probe to the frontal cortex or dorsal hippocampus of the chloral hydrate-anaesthetized animals. Citalopram (treatment for 14 days) given via the dialysis probe caused a more marked increase in 5-HT release (compared to the saline-treated control animals) when administered to the dorsal hippocampus but not when injected into the frontal cortex. Basal 5-HT release (prior

to the injection of citalopram) and the inhibitory effect of RU 24969 or CP-93,129 on 5-HT release (after injection of citalopram) did not differ between citalopram- and saline-treated animals in both brain regions.

The possibility that *lithium* given for up to 21 days affects the sensitivity of the inhibitory 5-HT autoreceptor was also examined. After administration of lithium to rats in the food for 3 weeks, the sensitivity of the inhibitory 5-HT autoreceptor in slices of the cerebral cortex, hippocampus and hypothalamus was decreased (Friedman and Wang 1988, Wang and Friedman 1988). When rats were injected i.p. with lithium for 3 days only, the sensitivity of the autoreceptor was decreased in hippocampal, but not in cortical slices (Hotta and Yamawaki 1988). However, a shortcoming was that in the former two studies, the rats received food pellets containing lithium until sacrifice and in the latter, the last injection of lithium was administered only 1 h before sacrifice. Thus, lithium was probably not yet washed out of the tissue. This is a drawback since lithium, directly applied to the tissue in superfusion experiments, was shown to abolish the inhibitory effect of exogenously added 5-HT on 5-HT release (Hide and Yamawaki 1989).

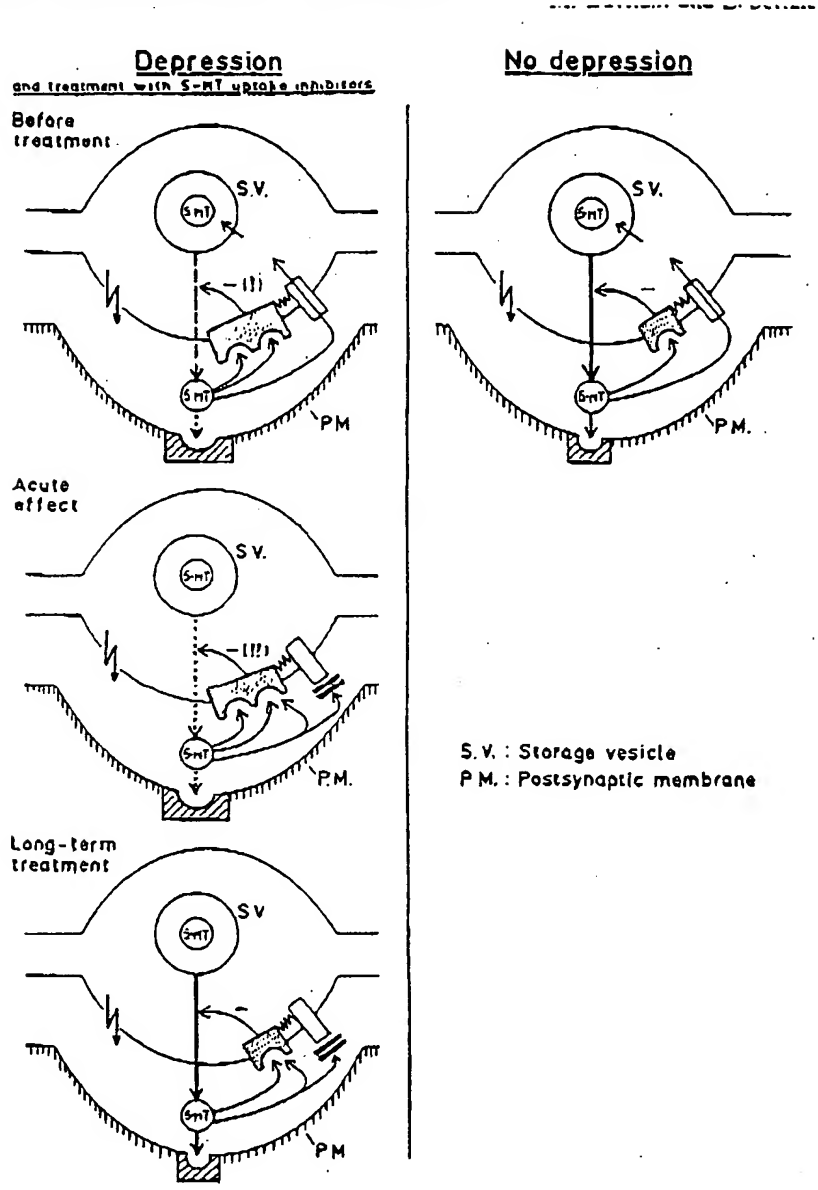
Taken together, evidence in support of the view that the sensitivity of the inhibitory presynaptic 5-HT autoreceptors is modified by long-term treatment with drugs used in affective disorders is equivocal.

Whether or not long-term treatment with such drugs may affect the sensitivity of 5-HT receptors mediating *facilitation* of 5-HT release has almost not yet been investigated at all. In the only study available, pretreatment of guinea pigs with paroxetine 10 mg/kg for 3 weeks decreased the facilitatory effect of 2-methyl-5-HT on the electrically evoked 5-HT overflow in frontal cortical, hippocampal and hypothalamic slices (Blier and Bouchard 1994).

G. Potential Pathophysiological and Therapeutic Roles of Inhibitory Presynaptic 5-HT Autoreceptors

Since 5-HT is involved in the central regulation of blood pressure (for review, see Wolf et al. 1985; Coote 1990), it was conceivable that alterations of inhibitory 5-HT autoreceptors in the CNS might play a role in the pathogenesis of hypertension. Therefore, the sensitivity of these receptors in the hypothalamus, nucleus tractus solitarii and cerebral cortex of spontaneously hypertensive (SHR) was compared to that in age-matched Wistar-Kyoto (WKY) rats (Schlicker et al. 1988). However, irrespective of the age of the rats and the brain region, neither 5-HT release in the absence of drugs nor modulation by exogenous 5-HT and methiothepin differed between the two strains.

5-HT probably plays an even more important role in various other functions of the CNS, including modulation of pain, feeding behaviour, neuroendocrinology, sleep, locomotor activity, sexual activity, mood and cognitive function. Furthermore, psychiatric and neurological diseases such as depres-


Depression
and treatment with 5-HT uptake inhibitors
No depression
Before treatment
Acute effect
Long-term treatment
S.V.
5-HT
PM
S. V. : Storage vesicle
P M. : Postsynaptic membrane

sion, anxiety, obsessive-compulsive disorder and myoclonus may be caused or accompanied by alterations in the serotoninergic system (for review, see SIEVER et al. 1991). In particular, reduced serotoninergic neurotransmission has been hypothesized to be involved in the pathogenesis of major depression (COPPEN 1967). It is conceivable that a decrease in 5-HT release is due to an increased sensitivity of the inhibitory presynaptic h5-HT_{1B} autoreceptors (GÖTHERT 1991; GÖTHERT and SCHLICKER 1993; CHOPIN et al. 1994; see Fig. 3).

This view is supported in an animal model of depression, namely the learned helplessness paradigm test in rats (for detailed discussion see CHOPIN et al. 1994; Chap. 10, this volume). The depression-like state of learned helplessness was induced by prolonged exposure of rats to repetitive electrical footshocks from which the animals could not escape. As a symptom of the depression-like state, it was observed that the rats reacted apathic and did not try to escape from this stress, when, subsequent to the "training session" with unescapable shocks, they had the opportunity to avoid the footshocks. This depression-like state of learned helplessness was associated with an upregulation of 5-HT_{1B} receptors, as suggested by an increase in density of [^{125}I]cyanopindolol-binding sites in the cortex, hippocampus and septum (EDWARDS et al. 1991). Such an upregulation may refer not only to postsynaptic 5-HT_{1B} receptors on the somadendritic or terminal areas of non-serotoninergic nerves but also to the presynaptic 5-HT_{1B} autoreceptors. In agreement with an increased responsiveness of presynaptic 5-HT_{1B} autoreceptors resulting from such an upregulation, a decrease in 5-HT release has been measured in microdialysis studies in depressed-like learned helpless rats (PETTY et al. 1992).

Fig. 3. Function of the inhibitory presynaptic 5-HT_{1B} autoreceptor on a serotoninergic varicosity in the brain under normal conditions (*right panel*) and hypothesized function in depressed patients before and after treatment with selective or non-selective 5-HT uptake inhibitors (*left panel*). *Stippled area* in the membrane of the serotoninergic varicosity, 5-HT_{1B} autoreceptor; *V*, action potential. *Solid vertical arrows*, normal 5-HT release resulting in normal 5-HT concentration at postsynaptic 5-HT receptors; *broken vertical arrows*, decreased release and decreased 5-HT concentration at postsynaptic 5-HT receptors; *thin solid arrows* within the synaptic cleft, binding of 5-HT to presynaptic autoreceptors and 5-HT uptake by the 5-HT transporter (*rectangle* within the cell membrane). Autoreceptor-mediated inhibition of 5-HT release: -, normal inhibition; -(!) and -(!!), reinforced inhibition. In untreated, depressed patients, the number of autoreceptors is assumed to be increased, symbolized by the increased *stippled area*, by the two recognition sites for 5-HT and by -(!). Blockade of the transporter by certain antidepressants (!!) initially results in an increased concentration of 5-HT at the autoreceptor, leading immediately to further reinforcement of autoreceptor function, symbolized by -(!!). As a result of both effects (uptake blockade and increased autoreceptor function), the 5-HT concentration at postsynaptic receptors remains decreased. After long-term treatment with such antidepressants, downregulation of the autoreceptor may occur, resulting in normal serotoninergic synaptic transmission. *Hatched area* in the postsynaptic membrane, non-5-HT_{1B} (e.g. 5-HT_{2A}) receptor. (From GÖTHERT and SCHLICKER 1995)

On the other hand, a supersensitivity of the postsynaptic receptors may be associated with an increased level of anxiety which has basically been related to an elevated serotoninergic "activity" (CHOPIN and BRILEY 1987). The neurons innervated by serotoninergic axon terminals and endowed with an increased density of postsynaptic 5-HT_{1B} receptors may be hyperstimulated even when, due to the simultaneous supersensitivity of presynaptic 5-HT_{1B} autoreceptors, the release of 5-HT is to a certain, yet relatively less, extent decreased. In agreement with these considerations and the results of the binding studies mentioned above, learned helpless rats exhibited behavioural signs of anxiety (VANDIJKEN et al. 1992a,b) combined with depression-like symptoms (SHERMAN et al. 1979). Analogously, supersensitivity of pre- and postsynaptic 5-HT_{1B} receptors in man would be expected to be associated with depression and anxiety, respectively, a combination which is frequently observed in patients.

It is well known that the therapeutic effect of antidepressant drugs (tricyclics, SSRIs) develops within 2–3 weeks of treatment whereas the blockade of the 5-HT (and noradrenaline) transporter(s) occurs immediately. The reason for this discrepancy probably is that not the inhibition of the transporter(s) per se but rather adaptive changes of pre- and postsynaptic receptors, including the presynaptic 5-HT_{1B} autoreceptors (see Fig. 3 and Sect. F.III in this context), account for the therapeutic effect. In depressed patients the presynaptic 5-HT_{1B} autoreceptors may be assumed to be supersensitive and, as a consequence, release of 5-HT from the serotoninergic axon terminals may be hypothesized to be diminished. Under these conditions, acute administration of a drug which inhibits 5-HT reuptake does probably not increase serotoninergic neurotransmission: the decreased inactivation of 5-HT may be assumed to lead to an initial increase in 5-HT concentration in the synaptic cleft which, in turn, causes an increased stimulation of the inhibitory autoreceptors and, as consequence, a diminished 5-HT release (GÖTHERT 1991; GÖTHERT and SCHLICKER 1993). Thus, these opposite effects may outweigh each other (Fig. 3). However, after long-term treatment, the sensitivity of the presynaptic 5-HT autoreceptors may decrease, leading to an increase in 5-HT release. This hypothesis is supported by some (although not all) studies in experimental animals which revealed a downregulation of the presynaptic 5-HT autoreceptors and an increase in 5-HT release after administration of antidepressant drugs for 2–3 weeks (see Table 4 and Sect. F.III). An additional mechanism may contribute to the increase in 5-HT release, namely the desensitization of the inhibitory somadendritic 5-HT_{1A} autoreceptors (for reviews, see BLIER and DE MONTIGNY 1994; GARDIER et al. 1996).

On the basis of the hypothesis that an increased serotoninergic neurotransmission relieves certain symptoms of depression, blockade of inhibitory somadendritic or presynaptic 5-HT autoreceptors, leading to an immediate increase in 5-HT release (for in vitro evidence for such an effect, see Fig. 1), has become an attractive concept for the development of novel antidepressant drugs (GÖTHERT 1982, 1991; GÖTHERT and SCHLICKER 1993; ARTICAS 1993; BRILEY and MORET 1993a,b; BLIER and DE MONTIGNY 1994;

CHOPIN et al. 1994). Such h5-HT_{1A} or h5-HT_{1B} receptor antagonists, respectively, in particular when combined with an inhibitor of the 5-HT transporter, may be assumed to exhibit the advantage of inducing an immediate antidepressant effect without the latency period characteristic for the currently available antidepressant drugs. In fact, clinical evidence has been obtained for an increased onset of antidepressant effect when an SSRI was combined with the h5-HT_{1A} receptor antagonist pindolol (ARTIGAS et al. 1994; BLIER and BERGERON 1995). In agreement with this effect of somadendritic autoreceptor blockade, it has recently been proven in an in vivo microdialysis study in guinea pig hypothalamus (ROLLEMA et al. 1996) that blockade of presynaptic 5-HT_{1D} autoreceptors by GR 127935 [(N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide)] potentiates the weak increasing effect of sertraline, an SSRI, on 5-HT release.

H. Concluding Remarks

Inhibitory presynaptic 5-HT autoreceptors have been shown to play a physiological role in the regulation of 5-HT release in the central nervous system. They have been identified in any region of the mammalian brain and spinal cord which has been investigated for this purpose. Furthermore, 5-HT release in the guinea pig and rat brain cortex, hippocampus and hypothalamus is modulated via facilitatory 5-HT receptors. These are not autoreceptors but may be assumed to be located on an interneuron which is involved in a neuronal circuit impinging on the serotoninergic axon terminals. The latter terminals should be endowed with a heteroreceptor for a neurotransmitter released from the innervating interneuron.

Whereas the facilitatory 5-HT receptors, whose physiological role in the regulation of 5-HT release is uncertain, belong to the 5-HT_3 receptor class, the inhibitory 5-HT autoreceptors have been classified as 5-HT_{1B}. The latter receptors differ considerably in their pharmacological properties among various species, e.g. between rat and mouse on the one hand and guinea pig, rabbit, pig and man on the other. Accordingly, guinea pig cerebral preparations have proven to represent excellent models for the development of selective ligands at inhibitory presynaptic 5-HT autoreceptors in the human brain.

As shown in some, but not all, animal experiments, long-term treatment with tricyclic antidepressants and SSRIs, both of which inhibit neuronal 5-HT uptake, induces a downregulation of the inhibitory presynaptic 5-HT autoreceptors. By attenuating the autoinhibitory feedback loop this may be assumed to result in an increase in serotoninergic neurotransmission, thus probably contributing to the mechanism of the antidepressant action of those drugs.

In view of the marked heterogeneity of 5-HT receptors and their specific distribution and function in the human CNS, the classification of the inhibitory 5-HT autoreceptors as h5-HT_{1B} offers a promising chance for the development

of drugs with beneficial effects in certain neuropsychiatric disorders. Thus, selective antagonists at human 5-HT_{1B} receptors, which immediately and considerably increase the release of 5-HT, may be expected to possess more favourable properties as, e.g. antidepressant drugs than the available ones. This may refer to their main actions in relation to potential side effects and to a more rapid onset of action.

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